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CONTENTS

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VOLUME 4 MAY, 1944 NUMBER 5

Experimental Brain Tumors

V. Behavior in Intraocular Transplants*

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(Received for publication December 20, 1943)

Two considerations prompted the performance of the experiments that will be detailed in this communication. One was the desirability of studying the growth and behavior of transplants of a variety of chemically induced brain tumors in mice from their early inception to maturity. The anterior chamber of the eye was selected as the most favorable site for these experiments because the transparent cornea affords an opportunity to keep the transplants under constant observation, even with the aid of a microscope when necessary.

The second consideration concerned itself with the question whether these experimentally induced brain tumors are genuinely malignant or, at least, whether they have one of the important characteristics of malignancy; namely, self-sufficiency or autonomy. The concept of autonomous growth as evidenced by the ability of tumor tissue to survive in a foreign environment such as the anterior chamber of the eye has been elaborated by Greene (3, 4). It is perhaps doubtful whether this criterion of malignant tumors is of greater diagnostic value than anaplasia, local invasiveness, distant metastasis, or an impairment of the cocarboxylase enzyme system (1). Previous reports dealing with primary brain tumors induced experimentally (2, 5, 6) have emphasized the local invasive proclivities of these neoplasms and, in the case of certain of the gliogenous tumors, have shown the tendency to metastasize along the spinal fluid pathway. But none of the tumors, especially the gliomas, ever metastasized extracranially, a behavioral characteristic shared by human gliomas as well. To be sure, many of the new growths displayed striking evidence of anaplasia, but it was felt that if these tumors showed the property of autonomous growth in the anterior chamber of the eye their malignant nature would be established beyond reasonable doubt.

* This investigation was aided by a grant from The Jane Coffin Childs Fund for Medical Research.

METHODS

The brain tumors employed in these transplantation experiments were all induced with 20-methylcholanthrene in C3H and Bagg albino mice. The animals into whose anterior eye chambers the transplants were made were C3H, dba, and ABC albino mice, as well as guinea pigs.

Technic of tumor implantation in the mouse's eye.— A suspension of tumor cells was prepared in the usual manner. The finest hypodermic needle, 27 gauge, ¼ inch length, fitting the ordinary type Luer tuberculin syringe, was employed. It was found that explants measuring 0.5 mm. in diameter could easily pass through a needle of this gauge. If clumps of tumor cells became packed near the exit opening of the syringe, increased pressure merely formed larger compact masses that clogged the needle. In an attempt to avoid this difficulty the opening of the ordinary tuberculin syringe was reamed out to a diameter of 2 mm. However, the same difficulty sometimes occurred within the needle base, where it tapers sharply. By withdrawing the suspension from the needle immediately after each injection and constantly agitating the syringe these difficulties could be avoided.

It was necessary to hone the needle to an exceptionally sharp point in order to penetrate the almost spherically convex cornea with minimal trauma. This was done by placing a small oil stone on a dissecting microscope stage and under observation passing the needle, fitted to a small syringe without the plunger, lightly along the stone. The syringe was held at about 15° to the upper surface of the stone and was twirled between the thumb and first two fingers so that it made a complete revolution with each motion. A stylet was inserted in the needle and was allowed to extend just to the needle tip and no farther. Its presence prevented wobbling due to the bevel of the needle, and uneven honing. About 10 or 12 double passages

across the stone were found sufficient, each additional stroke tending to spoil the point. Honing with jeweler's lathes and dentist's wheels gave inferior results.

Mature mice were preferred because the eyes are larger. Light ether anesthesia was chosen in preference to nembutal since the animals recover more quickly from its effects, and the narcosis can be extended by a few drops on a wisp of cotton. The mouse was placed on a small board on the stage of a dissecting microscope with a small spot of strong light focused on the eye. The left eye was selected for right-handed operators. The animal's head was steadied between the index finger and thumb of the left hand, the vibrissae were clipped, and extremely gentle pressure was made against the base of the occiput with the left middle finger, causing the left eye to bulge outward slightly. Too great pressure caused intracranial trauma. The microscope, with a magnification of about 10 diameters, was focused on the iris. An assistant, having the syringe and suspension prepared, handed the syringe, with the bevel up, to the operator.

The point of the needle was introduced at the limbus, on a line with the external canthus. Gentle pressure was made until the entire opening of the needle was within the anterior chamber. While the syringe was steadied by the operator, pressure was made on the plunger by the assistant. The operator controlled the amount of injection by signalling to stop when several fragments of tumor entered the anterior chamber. The needle was then deliberately and quickly removed at the same time that the pressure against the head was released. Nothing was gained by injecting a large mass into the eye and distorting normal relations.

Because the anterior chamber of the mouse's eye is very shallow, there was difficulty in avoiding the lens and iris. Injection of some of the tumor material into the iris, lens, and even the vitreous body often took place. It was found, however, that this seeding of tumor cells in the various structures was an actual advantage since their extension from the posterior into the anterior chamber by way of the pupil afforded excellent material for study.

The injected eye was first examined 24 hours after operation. For this procedure an ophthalmologist's (Gullstrand) slit-lamp and microscope were used. It was not necessary to anesthetize the animal for daily examinations since sufficient manual immobilization was possible for a magnification of about 20 diameters. Higher magnifications necessitated anesthesia.

Loss of eyes from infection was not experienced. Slight corneal cloudiness, traumatic cataract, anterior and posterior synechiae, hyphemia, and other changes were occasionally seen, but these changes did not appear to interfere with tumor growth.

Technic of tumor implantation in the guinea pig's eye.—The suspensions of the primary mouse brain tumors were prepared for intraocular implantation in the guinea pig in a manner similar to that for the mice. Under nembutal anesthesia the cornea of the left eye was nicked with a scalpel to aid the introduction of an ordinary 27 gauge needle. In general, both larger total amounts and larger individual pieces of brain tumor tissue were introduced into the anterior chambers of the eyes of these animals than into those of the mice. In all other respects this animal species was treated and studied in a manner strictly comparable to that employed for the mice.

RESULTS

The experimental brain tumors employed in these intraocular transplantation experiments were induced with 20-methylcholanthrene in C3H and Bagg albino mice. They have already been reported in some detail elsewhere (5, 7) and hence reference to their microscopic appearances will be brief in this report. Emphasis, rather, will be placed here on the characteristics of their growth behavior in intraocular transplants. For comparative purposes the appearances of certain extracranial sarcomas will also be described. Finally, details will be given of the intraocular growth of a human glioblastoma multiforme.

Astrocytoma.—The material for transplantation into the anterior chamber of the eye was derived from mouse 69 and was described in the paper on tumors produced with methylcholanthrene (5), where its morphologic features were illustrated in Fig. 13. Four C3H and an equal number of ABC albino mice received intraocular transplants of this neoplasm. After a period of quiescence that varied from 10 to 19 days, the tumor began to grow in the anterior chambers of the C3H mice, filling them completely by the 35th day. There was invasion of the posterior chambers through the pupils, and the iris and cornea of each animal were likewise infiltrated by the neoplasm. An outstanding characteristic of this tumor was its ivory white color and the almost complete lack of vascularity and hemorrhage (Fig. 1). Microscopically the tumor cells had the same characteristics as those of the primary neoplasm, even as regards astrocytes in mitotic division.

The tumor transplants behaved quite differently in the ABC albino mice. Within a week all 4 animals showed evidence of active tumor growth, then recession began so that the eye was clear in 9 days in the first mouse, 15 days in the second and third mice, and 32 days in the fourth mouse.

Medulloblastoma.—The brain tumor employed in this experiment came from mouse 49 and was illustrated in Fig. 4 of the paper on experimentally induced

brain tumors with methylcholanthrene (5). Intraocular transplants were made into 5 C3H, 4 ABC albino, and 3 dba mice. All animals of the first strain showed active tumor growth in an average of 14 days. The anterior chambers became filled with the neoplasm, which invaded and destroyed the irides. The posterior chambers then became filled by extension through the pupils. Ultimately there was invasion of the vitreous body, the cornea, and, in at least one instance, the retina as well. Melanin pigment granules appeared in the solid milky white tumors after the irides were invaded. Delicate blood vessels developed in the transplants after these had reached a considerable size (Fig. 2).

Initially, all 4 ABC albino mice showed evidence of growth of the transplants in about 2 weeks, but shortly thereafter the tumors disappeared from the eyes of 2 of the animals. The remaining 2 mice, however, had vigorously growing transplants until the animals were killed in order to permit microscopic study of the neoplasms. The appearance of both intraocular tumors was similar to that in the C3H mice.

Only 1 of the dba mice receiving intraocular transplants of the brain tumor showed any signs at all of tumor growth in the anterior chamber. A month elapsed before the transplant increased in size appreciably, but ultimately a solid milky white mass of tissue filled half of the anterior chamber and extended through the pupil into the posterior chamber.

The intraocular tumor from 1 of the original 5 C3H mice was employed for subtransplantation into 4 C3H and 4 ABC albino mice. One animal of the first group and 2 of the second had takes. All 3 subtransplants were growing actively by the 12th day. In the C3H mouse the tumor again grew as a solid milky white mass; in the ABC albino animals the tumors were finely granular but also milky white in color.

The microscopic appearance of all the individual transplants was essentially identical. The tumor cells were somewhat elongated, not unlike fibroblasts, but there was practically no stroma. Many of the cells were in mitotic division. Frequent pseudorosette formations helped identify the transplants as medulloblastoma.

Ependymoblastoma.—Two guinea pigs received intraocular transplants from a brain tumor induced in a Bagg albino mouse with methylcholanthrene (mouse 20). This animal was included in a report on the incidence of experimentally induced brain tumors in different strains of mice (7). The tumor was a typical ependymoblastoma with numerous rosettes and was similar to the tumor of this variety illustrated in Fig. 2 of the communication dealing with brain tumors induced with benzpyrene (6). Within 2 days after intraocular transplantation the tumor showed signs of growth in both guinea pigs' eyes. Within 4 days the flocculent tumor material extended over much of the irides and became implanted on the anterior surface of the lens capsules. Individual tumor nodules began to coalesce and form larger grey masses, which received their vascular supply from delicate vessels arising at the inferior angle of the anterior chamber (Fig. 3). The transplants continued their active growth for 20 days until the eyes were removed for microscopic study. This disclosed a rapidly growing tumor, as indicated by the presence of numerous cells in mitotic division, with many true rosettes.

Unclassified cerebral glioma.—The primary brain tumor was derived from mouse 74 and presented considerable diagnostic difficulty, which has been discussed elsewhere (5). This neoplasm was a highly malignant glioma with many features suggestive of a glioblastoma. Its histologic details were portrayed in Fig. 12 of the article mentioned above. Five C3H and 4 ABC albino mice received transplants of this tumor. These transplants grew in only 1 animal of the C3H and in 2 of the ABC albino strain. After 2 weeks of quiescence active growth was recognized by the appearance of discrete milky white nodules, which soon began to fuse and become highly vascularized. All the intraocular structures were invaded by the neoplasm and hemorrhages, some of fair size, began to appear as the eyeball became greatly enlarged. When rupture threatened, the eyes were removed for microscopic study.

The original brain tumor had been transplanted subcutaneously in the homologous strain of mice, where it continued to grow through 12 generations of subtransplants. Pieces of this tumor derived from the third generation of subcutaneous transplant were again implanted in the eyes of 3 C3H mice, and 1 animal developed a take. This occurred within 4 days after implantation. Growth proceeded rapidly and went through the stages already described for this tumor (Fig. 4).

The microscopic appearance of all 4 intraocular transplants of this neoplasm was similar. There was great cellular pleomorphism with many cells in mitosis. The stroma was scant and none of it was of mesodermal origin.

Unclassified cerebellar glioma.—The brain tumor that was induced in a C3H mouse (mouse 42) with methylcholanthrene was transplanted into the anterior chambers of the eyes of 4 C3H and 4 ABC albino mice. This was a cerebellar glioma that was difficult to classify since the tumor cells failed to form characteristic patterns. Two of the C3H mice showed evidence of growth in the transplants after 21 days, the tumors first filling the anterior chambers with solid milky white material, then invading the posterior chambers, later the vitreous body and lens capsules, and finally

rupturing the corneae. Growth proceeded from that stage to form large epithelialized masses that projected from the orbits (Fig. 5). Microscopic examination of the tumors revealed a core of gliogenous tissue surrounded by a scant amount of connective tissue, which served as the basement membrane for a stratified squamous epithelial covering. As in the original tumor, these transplants failed to form histologic structures that would warrant a diagnosis of more than glioma. The other 2 C3H mice never showed signs of tumor growth.

Three of the ABC albino mice appeared promising from the standpoint of transplant takes after some 3 weeks, but later all 3 tumors regressed rapidly. The transplanted material in the fourth animal showed no signs of growth from the start.

Cerebellar fibrosarcoma.—The primary brain tumor was induced with methylcholanthrene in the cerebellum of a C3H mouse (mouse 145). It arose apparently from the leptomeninges overlying the vermis and invaded both lateral cerebellar lobes. It consisted of elongated, spindle-shaped cells, many in division, and had an abundant stroma of mesodermal reticulum. Transplants of this tumor were made into the anterior chambers of the eyes of 15 C3H mice and 13 of the tumors were actively growing at the end of 6 days. The remaining 2 animals failed to develop takes. In all 13 positive mice the new growths assumed characteristics that distinguished them from any of the gliomas of the previous experiments. The tumor became highly vascularized almost at once and large hemorrhages appeared. There was much necrosis of the new growth, which became deeply pigmented in scattered zones because of the release of melanotic pigment from the irides. There also appeared to be a genuine proliferation of melanophores. These cells intermingled with the malignant elements of the fibrosarcoma. Within 2 weeks the eyeballs were replaced by tumor tissue and shortly thereafter the corneae ruptured. Extensive invasion of the orbital structures then followed, and in several of the mice that survived the adjacent cranial sinuses were invaded by the tumor.

Rhabdomyosarcoma.—The animal (mouse 9) whose tumor was employed for intraocular transplants developed a rhabdomyosarcoma of the scalp following intracerebral implantation of a pellet of methylcholanthrene. The pellet of carcinogen had worked its way out of the craniotomy wound and had come in contact with the temporal muscle, where the neoplasm originated. Nineteen C3H and 6 ABC albino mice were inoculated with this tumor and in all but 2 C3H animals active growth was established by the sixth day. These 2 mice, and 2 more of the same strain in which the tumors regressed after the tenth day, were the only animals that failed to be hosts to large intraocular neoplasms at the termination of this experiment on the 30th day. As in the case of the cerebellar fibrosarcoma described above, this rhabdomyosarcoma quickly developed an extensive vascular supply and soon after became hemorrhagic (Fig. 6). Parts of the tumor appeared gelatinous and parts granular with considerable brownish pigment. The latter was identified as melanin on microscopic examination and was absent only in the ABC albino mice (Fig. 7). The eyeballs were rapidly destroyed by the growing tumor, the corneae ruptured in all the animals that survived more than 3 weeks, and the orbital tissues were extensively infiltrated with tumor tissue.

An opportunity presented itself to study still another rhabdomyosarcoma induced in the scalp tissues of a C3H mouse with methylcholanthrene (mouse 60). Intraocular transplants of this neoplasm were made in 10 C3H mice, 6 of which developed takes. In these animals the eyes were completely filled with the growing tumor in from 21 to 30 days. In each, the new growth was characterized by much vascular proliferation and hemorrhage, as well as by an excess of melanin pigment deposition. The appearance of the tumor in the eyes of these animals was remarkably similar to the tumor derived from mouse 9.

A first generation subcutaneous transplant of this

DESCRIPTION OF FIGURES 1 TO 8

FIG. 1.—Astrocytoma. Transplant in anterior chamber of C3H mouse from primary brain tumor in mouse 69. Appearance 11 days after intraocular implantation.

FIG. 2.—Medulloblastoma. Appearance of tumor in C3H mouse 28 days after transplantation from mouse 49.

FIG. 3.—Ependymoblastoma. Appearance of tumor in guinea pig 13 days after intraocular transplantation from Bagg albino mouse 20.

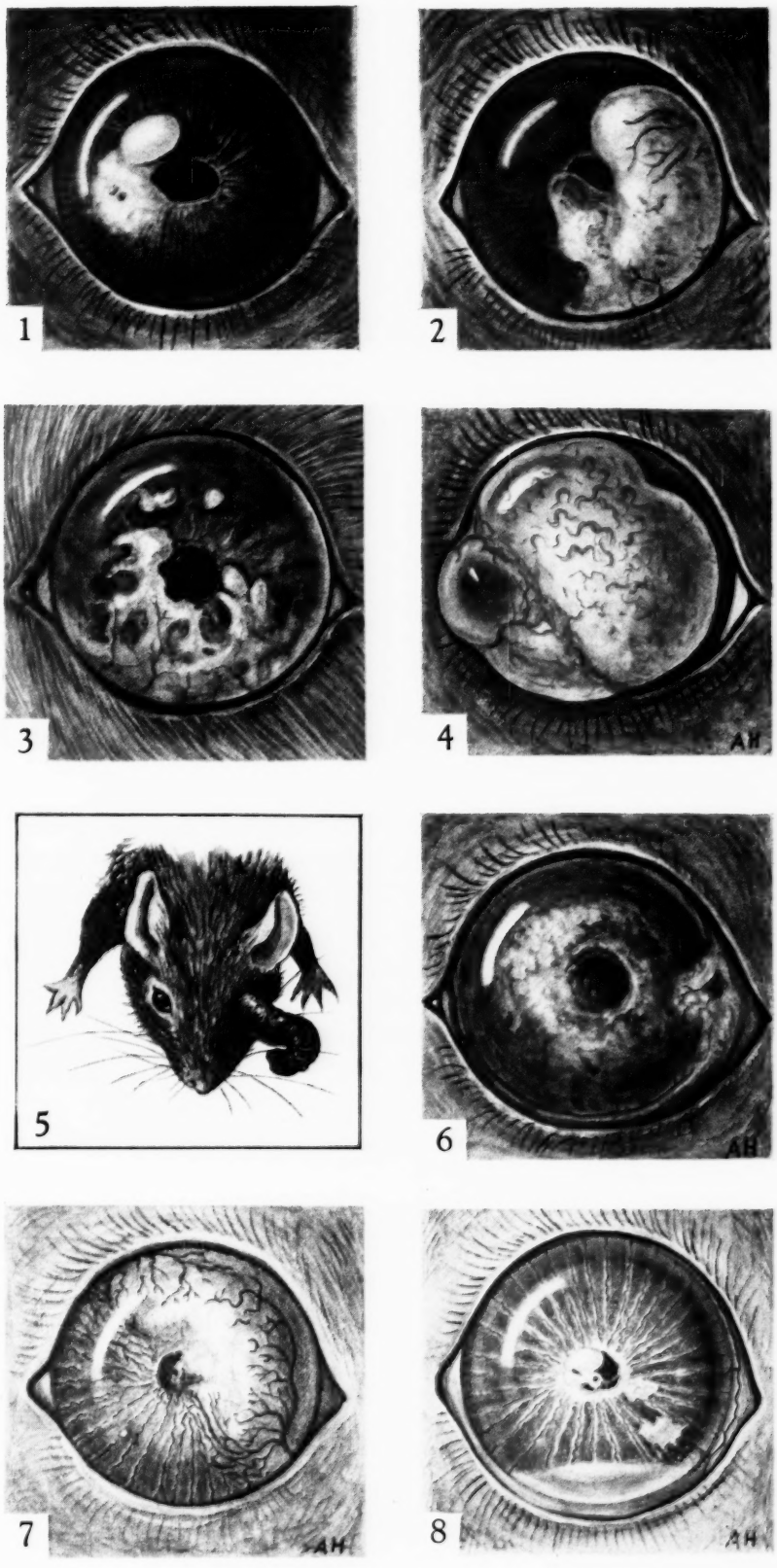
FIG. 4.—Unclassified cerebral glioma. Transplant in anterior chamber of C3H mouse. Tumor derived from mouse 74. Appearance 12 days after transplantation.

FIG. 5.—Unclassified cerebellar glioma. Extruded neoplasm 3 months after intraocular transplantation in C3H mouse. Primary tumor derived from C3H mouse 42.

FIG. 6.—Rhabdomyosarcoma. Transplant on seventh day of growth in eye of C3H mouse. Origin of primary tumor in animal of homologous strain (mouse 9).

FIG. 7.—Rhabdomyosarcoma. Vascularized transplant in ABC albino mouse on 18th day. Primary tumor same as that shown in Fig. 6.

FIG. 8.—Human glioblastoma multiforme. Appearance of transplant in anterior chamber of ABC albino mouse on 14th day. Primary tumor in 59 year old man.



FIGS. 1-8

rhabdomyosarcoma was utilized for subtransplantation intraocularly in 13 C3H mice. Growth of the tumor did not succeed in 2 of the animals, but the remaining 11 mice developed tumors that filled the eyes completely in from 17 to 32 days. Once again the transplanted tissue was characterized by extensive invasiveness, great vascularity, and hemorrhage.

Human glioblastoma multiforme.—This tumor was removed at operation from the right parietal lobe of a white man 59 years of age. It was a typical glioblastoma on microscopic examination (report No. N.P. 941). A portion of this neoplasm was prepared for intraocular transplantation into 4 C3H and an equal number of ABC albino mice. In 3 of the animals of the former strain the transplants were observed to grow well on the fifth day, but in the fourth animal regression began almost at once and was complete in 11 days. In 2 of the mice in which growth was obvious on the fifth day, regression began on the 17th day and was complete in 3 weeks. In the third animal regressive changes began on the tenth day and the anterior chamber was completely free of tumor at the end of 30 days. The tumor transplants were characterized by a milky white, avascular, and nonhemorrhagic appearance, and closely resembled gliogenous tumors induced in mice with methylcholanthrene.

Examination of the anterior chambers of the eyes of 2 of the ABC albino mice disclosed regression of the transplants, which began almost at once and was complete in 6 days in one and 14 days in the other animal. In the third mouse the entire anterior chamber was filled with tumor on the ninth day. The tumor had regressed somewhat by the 14th day (Fig. 8) and further still in the next 7 days, at the end of which the eye was removed for microscopic study. The fourth mouse revealed active growth of the transplant during the first 10 days; then regression began and was completed in 21 days. Microscopic study of the tumor in the third animal showed neoplastic cells in a wavy sheet lining the corneal and iris surfaces of the anterior chamber. Regressive changes, however, had proceeded to such a point that the characteristic histologic structure of a glioblastoma was not present in this transplant.

COMMENT

The expectations that prompted these experiments, namely, the opportunity to observe the growth characteristics of experimentally induced brain tumors by transplantation into the eyes of other animals and to establish that these tumors are capable of autonomous growth, have been fulfilled. An important difference was demonstrated in the appearance of the gliogenous tumors as compared with the sarcomas. Whereas the former class of tumors grew in large solid sheets of milky white color, the latter grew in clouds or minute flakes with much vascularity and hemorrhage. As regards these features, the more highly malignant gliomas occupied an intermediate position between the more benign gliomas, like the astrocytomas, and the sarcomas. At first they appeared as solid white masses which, when they increased in size, developed a moderately extensive vascularity and even a few small hemorrhages. With continued intraocular growth the gliomas eventually ruptured the cornea, whereas the sarcomas not only did this but destroyed the whole eyeball and continued to grow by replacing the orbital tissues and invading the adjacent cranial sinuses. Another striking difference between these 2 classes of neoplasms was the dispersion of much uveal melanin pigment in the sarcomas.

These experiments have established the fact that intraocular transplants of experimental brain tumors will grow about equally well in homologous and heterologous strains of mice. The failure of certain transplants to take must be ascribed in large measure to faulty technic. Of course, this explanation does not hold for those transplants that regressed after an initial period of active growth, but such occurrences were not limited to the heterologous strains. Regression was noted with almost equal frequency in the C3H and ABC albino mice. These experiments were not devised to contribute much of value to the problem of tumor regression.

Several observations of minor significance require comment. The occurrence of postoperative hemorrhage into the anterior chamber of the eye did not affect adversely the success or rapidity of growth of the transplants, nor did traumatic cataract seem to influence their growth. This can also be said of postoperative adhesions (anterior and posterior synechiae). Tumor cells frequently grew successfully on the inner surface of the cornea and on the lens capsule, but invasion of the lens itself was never observed. The meshwork of the iris angle, Schlemm's canal, and the retina were often invaded by tumor.

SUMMARY

A technic is described for mice and guinea pigs that permits the intraocular transplantation of brain tumors, those induced with a chemical carcinogen in mice as well as those occurring spontaneously in man. This method of study affords the opportunity of keeping the growing neoplasms under constant observation. It has demonstrated certain characteristics of neoplastic growth behavior and appearance that permit differentiation of gliomas from nongliogenous tumors. It has demonstrated, by the standard of autonomous growth

in homologous and heterologous strains of mice, that experimentally induced brain tumors represent true neoplasms.

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Influence of Environmental Temperature upon the Incidence and Course of Spontaneous Tumors in C3H Mice

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In a previous paper (4) we showed that tumors induced by subcutaneous injections of methylcholanthrene arise significantly earlier and grow faster in mice adapted to heat (91° F.) than in those kept at 68° F. Subcutaneous injections of emulsions of a spindle cell sarcoma also gave rise to tumors that grew rapidly in the heat, while in the 68° F. environment they grew very slowly or actually regressed. Such sarcoma emulsions injected intramuscularly, however, grew equally well in both hot-room and cold-room mice.

In 1941 Fuller, Brown, and Mills (3) reported a distinct difference of spontaneous tumor incidence in dba virgin females kept at 91° F. as compared to those living in a 68° F. environment. Not only was the number of tumors smaller in the heat, but they also appeared later in life and grew more slowly. That study was made on virgin dba females purchased at 6 weeks of age, with no litter-mate pairing between the hot and cold rooms. However, as this strain had a low tumor incidence, the number of tumors involved was not large, though the results were definitely significant.

In the present study our original breeders were obtained from Andervont's special subline of strain C3H mice (4) in January, 1941; careful inbreeding in our colony, however, has yielded only a 50 per cent incidence of mammary cancer in virgin females. Breeding of the mice was carried out in an air-conditioned room kept at 79° F., and at approximately 2 months of age the litters of young were segregated by sex and the females divided between the hot, cold, and control rooms. Purina dog chow checkers were used as the sole food throughout the study.

The mice were examined weekly for tumor appearance. Alternate tumor victims (considering each room separately) were sacrificed at the end of 1 month after the tumor was first noticed, in order that the rate of tumor growth might be estimated. All carcasses were slit open and preserved in 10 per cent formalin until time of examination and measurement of the mass. A number of the mice got a leg caught in the wire mesh floor of the cage and died or were killed; these were more numerous among the smaller hot-room mice and were not counted in the series. A minor typhoid or dysentery epidemic in one cage also caused a few early deaths, which were not included in the calculations. All possible efforts were made to base the final computations only upon proved tumor victims dying of the tumor (or killed at the end of one month) and nontumor mice not dying prematurely of accident or contagion.

All tumors were dissected out after formalin fixation of the carcass, and measurement was made of length, breadth, and thickness. These three dimensions (in millimeters) were multiplied together and the resulting numbers used as indices of tumor size. It is realized that such calculation does not represent the real tumor volume in any case, but its use seems justified for purely comparative purposes.

Table I presents the final data obtained on the series. Briefly stated, the significant findings are (a) a heightened tumor incidence in the cold-room animals, (b) a probably significant earlier age for tumor appearance in the cold, (c) a more rapid rate of tumor growth, and (d) longer duration of life after tumor appearance in animals exposed to the cold. Tumors appeared a month earlier in the cold than in the heat and grew almost twice as much in the first month after appearance. The difference in mouse age at tumor appearance (0.99 ± 0.42 months) is 2.3 times its own probable error and would occur by chance alone once in 8 times. The difference in indices of tumor size after 1 month's growth (3198 ± 773) is 4.14 times its own probable error and would occur only once in 200 times by chance alone.

Although the tumors grew more rapidly in the cold room, they seemed to kill the hot-room mice

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more quickly. Duration of life after tumor appearance was 48.08 days in the heat and 62.37 days in the cold, the difference (14.29 ± 5.31 days) being 2.7 times its own probable error. Such a difference would occur only once in 14 times by chance alone. A similar difference in tumor size at death was found when the mice were left to die naturally, but in this case the larger tumors of the cold room had been allowed a longer time for growth before the death of the animals.

In addition to the higher incidence, earlier appearance, and faster growth of tumors in the cold room, there was also a much more definite tendency for the development of multiple tumors in different breast segments of the same animal. In the hot room there were only 3 mice with 2 separate tumors each and one with 3; in the cold room, on the other hand, 14 mice developed 2 separate tumors each, 3 mice had 3, and 1 mouse showed 5 distinctly separate growths.

Table 1: Mammary Cancer Incidence in C3H Virgin Females Exposed to Heat and Cold

	At 68° F.	At 90-91° F. & 60-70% rel. hum.	Difference (68° vs. 90°)	Controls at 79° F.
Number of mice placed in rooms	125	122		75
Included in final calculations	114	96		48
Developing mammary tumors	82	48		24
Percentage incidence of mammary tumors	72	50	44% higher in cold	50
Mice with multiple tumors	18	4		2
Mean age (in months) at tumor appearance	13.34 ± 0.26	14.33 ± 0.34	0.99 ± 0.42	15.00 ± 0.62
Duration of life (in days) after tumor appearance	62.37 ± 4.25	48.08 ± 3.19	14.29 ± 5.31	———
Mean index of tumor size in mice killed 1 month after tumor appeared	7650 ± 514	4452 ± 577	3198 ± 773	4167 ± 685
Mean index of tumor size in mice left to die naturally	13710 ± 1107	8913 ± 1273	4797 ± 1687	———
Mean death age (in months) of nontumor mice	16.43 ± 0.55	16.52 ± 0.41	0.09 ± 0.68	20.75 ± 0.69

There was only one internal tumor found at autopsy, and that arose from the ovary of a hot-room mouse showing no mammary tumor.

Mean age at death of the nontumor mice was practically the same in the rooms kept at 68° F. and 91° F., but was later in the 79° breeding room.

DISCUSSION

In general this series of strain C3H mice yielded findings that fully verify the results previously obtained with the smaller group of dba virgin females. It seems evident that a cool environment stimulates spontaneous mammary neoplasia as compared to the effect of depressing heat upon this tendency in mice of special cancer strains. In a study still in progress, results already at hand indicate that this same difference exists in spayed females, in which case any ovarian influence upon mammary neoplasia is eliminated.

Our findings, previously published (4), also throw possible light upon climatic effects in skin cancer genesis. Actinic radiations from the tropical sun have been suspected by many as being one irritating factor concerned, but our hot-room mice show an increased susceptibility to skin cancer, either chemically induced or transplanted, in the absence of such actinic radiations. The answer would seem more likely to lie in an increased blood supply for heat-loss purposes and a more active cutaneous metabolism. In only one article, that by Bain, Rusch, and Kline (2), has the action of ultraviolet radiation been considered separately from the more general effects of radiant heat of all wave lengths. In their studies they found that filtered ultraviolet light had a definitely greater carcinogenic effect when environmental air temperatures were high (35° to 38° C.) than under ordinary laboratory conditions.

With cancer of the deeper tissues the situation is reversed, for such neoplasia shows definitely stronger tendencies in our cold-room mice. Although the mammary glands are located immediately under the skin, their blood supply and hormonal and nervous control caused them to be classed with the deeper tissues of the body in a metabolic sense. They are little affected, for instance, by the cutaneous vasoconstriction that takes place when the animal is chilled; rather, they are more likely to take part in the heightened metabolism that such chilling induces in the body as a whole.

Transplanted tumor cells develop poorly when injected into the subcutaneous regions of scant blood supply in cold-room mice, while very rapid growth results from their emplacement in the deeper and more active muscle tissues of the same animals. It would thus seem that one factor in cancer genesis is probably the blood supply and metabolic activity permitted under a given set of conditions. Environmental temperatures thus play their part in two ways: (a) by very definitely altering the blood supply to the skin for heat-loss purposes, and (b) by their slower effects upon metabolic activity in the deeper

tissues—stimulation in cool surroundings and depression in the heat. It should be borne in mind that the hot-room temperatures used here were just below the level required to produce fever in the animals. The development of thermic fever would alter the picture in many ways.

SUMMARY

1. Spontaneous mammary cancer in virgin C3H female mice shows the same increased incidence in cool environments that was previously found in virgin dba females.

2. These tumors appear 1 month earlier in life and grow faster at 68° F. than at 91° F., although they kill the hot-room victims more quickly.

3. Multiple tumors in this C3H mouse series were 4 times more frequent among the cold-room mice than among those kept in the heat.

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The Effect of Various Factors on the Harding-Passey Melanoma of the Mouse

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In 1925 Harding and Passey (4) discovered a transplantable mouse melanoma, the first recorded example of a transplantable melanotic tumor (4). In 1928 it was brought to this country from England by Dr. James B. Murphy, and since then has been continuously propagated at the Memorial Hospital. The tumor is intensely pigmented. It consists of large round cells and extraneous phagocytic cells full of melanin.

During the past 15 years this neoplasm has been successfully transplanted for 58 generations in more than 1,500 young albino mice. The transplants took in more than 95 per cent of the mice and regressed in about 3 per cent.

The grafts remained visible under the skin but lay nearly dormant for 3 weeks. Thereafter they usually showed a steady increase in size and might reach 50 mm. in diameter at the end of the 16th week. Many of the positive transplants in young animals (18 to 20 gm. body weight, or about 50 days old) continued to grow for about 100 days before ulceration of the tumor took place, and the subsequent death of the animal resulted usually from septicemia and nutritional failure (120 to 150 days). It would seem obvious that the tumors to be chosen for successful transplantation should be the most rapidly and progressively growing ones at about 45 days of age.

Metastases in the viscera did not occur, but much phagocyted melanotic pigment was found in the liver, spleen, and axillary lymph nodes when the organs of mice bearing tumors approximately 150 days old were examined histologically. On the other hand, very little melanotic pigment was found in the lungs and none in the kidney, adrenal, heart, and brain. Microscopic examination of the pigmented axillary lymph nodes revealed the presence of much phagocyted melanin from the primary tumors. Upon implantation, the pigmented axillary nodes failed to produce any new growths, an indication of their nonmalignant character. This agrees with the observation of Harding and Passey.

With this rare tumor available, it was thought of interest to determine the susceptibility of suckling mice; the effect of gonadectomy and ovariectomy on susceptibility to the growth; and to examine its behavior toward chemical and physical agents.

Transplantation into Suckling Mice

In man, melanomas may occur at any age but the incidence tends to increase with advancing years, probably because time affords opportunity for the malignant transformation of previously benign pigmented nevi (13).

Our previous studies with transplantable rat tumors indicated clearly that age had a definite influence upon the continued growth of transplants (16). In suckling rats, whose ages varied from less than a day to 20 days, implantation of the Sugiura rat sarcoma was successfully performed and the resulting tumors grew progressively until ulceration. Spontaneous absorption occurred in only 9 per cent of these young rats. On the other hand, the percentage of takes in old rats (about 1 year old) was the same as in suckling rats, but the tumor regressed in 88 per cent of the animals. In the case of the Flexner-Jobling rat carcinoma, the age of the animals has very little influence upon spontaneous absorption. This is shown by the fact that in suckling, young adult, and old rats regression was found to be about 15 per cent of the positive transplants in all three groups.

The study was extended to include the Harding-Passey mouse melanoma to ascertain whether there was any difference in susceptibility, growth rate, and spontaneous absorption at various ages.

Grafts weighing from 1 to 2 mgm. were selected from a healthy area of a tumor from 5 to 7 weeks old and inoculated subcutaneously into the lateral thoracic region of suckling mice with a small trocar, the diameter of which was approximately 1.5 mm. The sucklings were allowed to remain with their mothers until the age of 21 days. The animals were fed a normal diet of Purina dog chow and carrot, with as much fresh water as they would take.

Table I gives the results obtained from transplanting the pieces of tumor tissue into suckling mice

whose ages varied from less than a day to 21 days. The data are arranged according to the increasing ages of the animals.

The data indicate clearly that age of the host had no definite influence upon the continued growth of transplanted mouse melanoma. The implants were generally successful (average takes, 87 per cent) and grew progressively to the size of a walnut. Male and female suckling mice were found equally susceptible; of the 164 employed, 77 were males and 87 females. The transplants grew very slowly, however, during the first 3 weeks, as did those in young adult or old mice; they proliferated more rapidly thereafter for 4 to 6 months and subsequently death of the animal usually resulted from toxemia, septicemia, and nutritional failure. About 3 per cent of the positive grafts regressed spontaneously. Tumor metastases did not occur in the viscera.

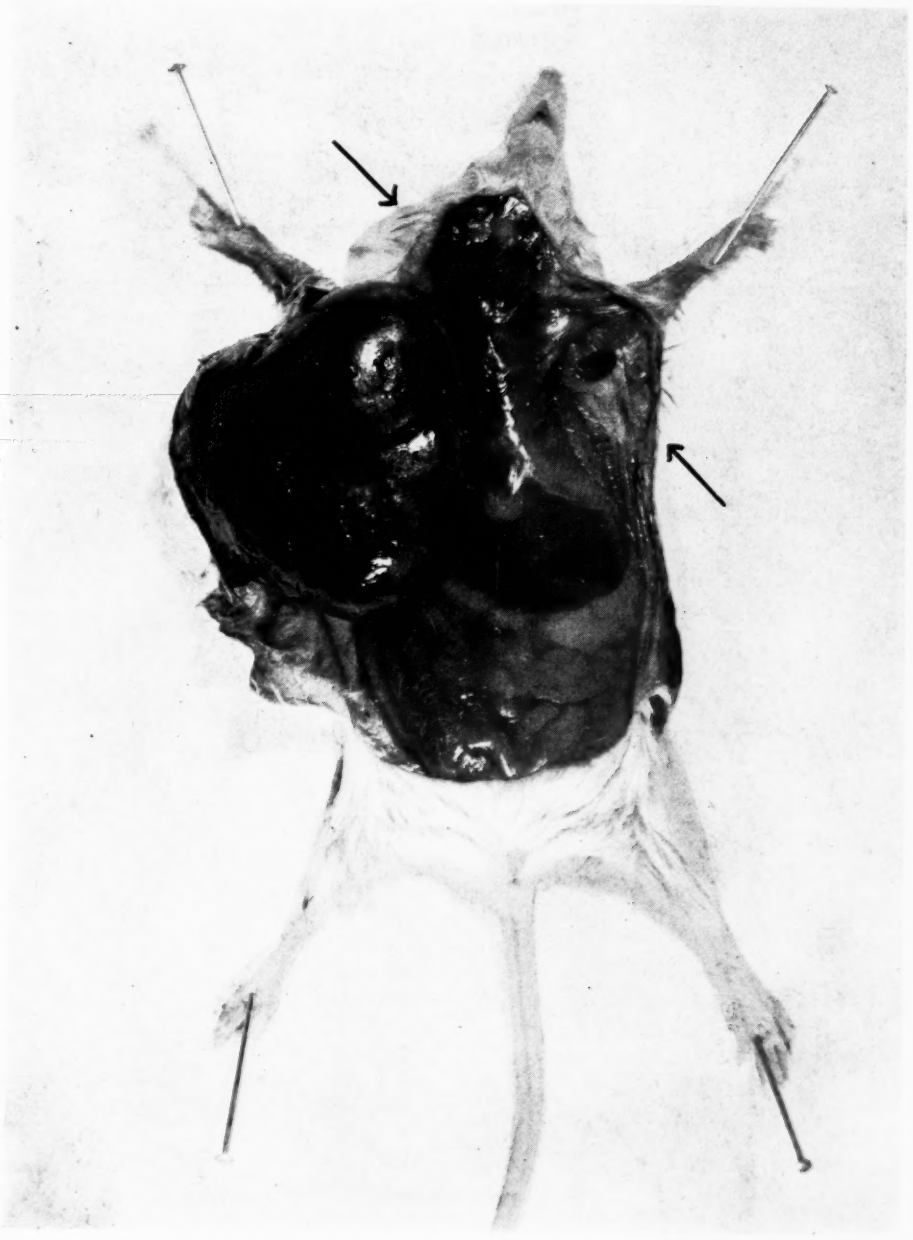

FIG. 1.—Intensely pigmented Harding-Passey mouse melanoma, 100 days old. One arrow points to a deeply pigmented axillary lymph node, the other to a cervical node.

CASTRATION AND TUMOR GROWTH

Several investigators have already reported the effect of castration on subsequently transplanted carcinomas or sarcomas. Lathrop and Loeb (9), Strong (15), Murphy and Sturm (11), Loeper and Turpin

(10), Cori (1), and Murray (12) have all reported that castration before puberty definitely lowered susceptibility in mice. On the contrary, Sweet, Corson-White, and Saxon (21); Joannovics (6); and Kamikawa and Kawamura (7) reported a considerable increase in susceptibility both as to the number of takes and the growth rate of tumors in castrated animals. Graf (3) found that castration in males and females had no influence on the growth of transplanted tumors. Recently Woglom (23) reviewed 17 papers on the influence of castration upon the neoplastic effects of tar or the synthetic carcinogenic agents. Some investigators found that castration inhibited, while others found a stimulating effect, and still others that the operation had no effect upon the development of malignant tumors. Woglom himself found that prepuberty castration neither augmented nor diminished the susceptibility to methylcholanthrene sarcoma in male mice.

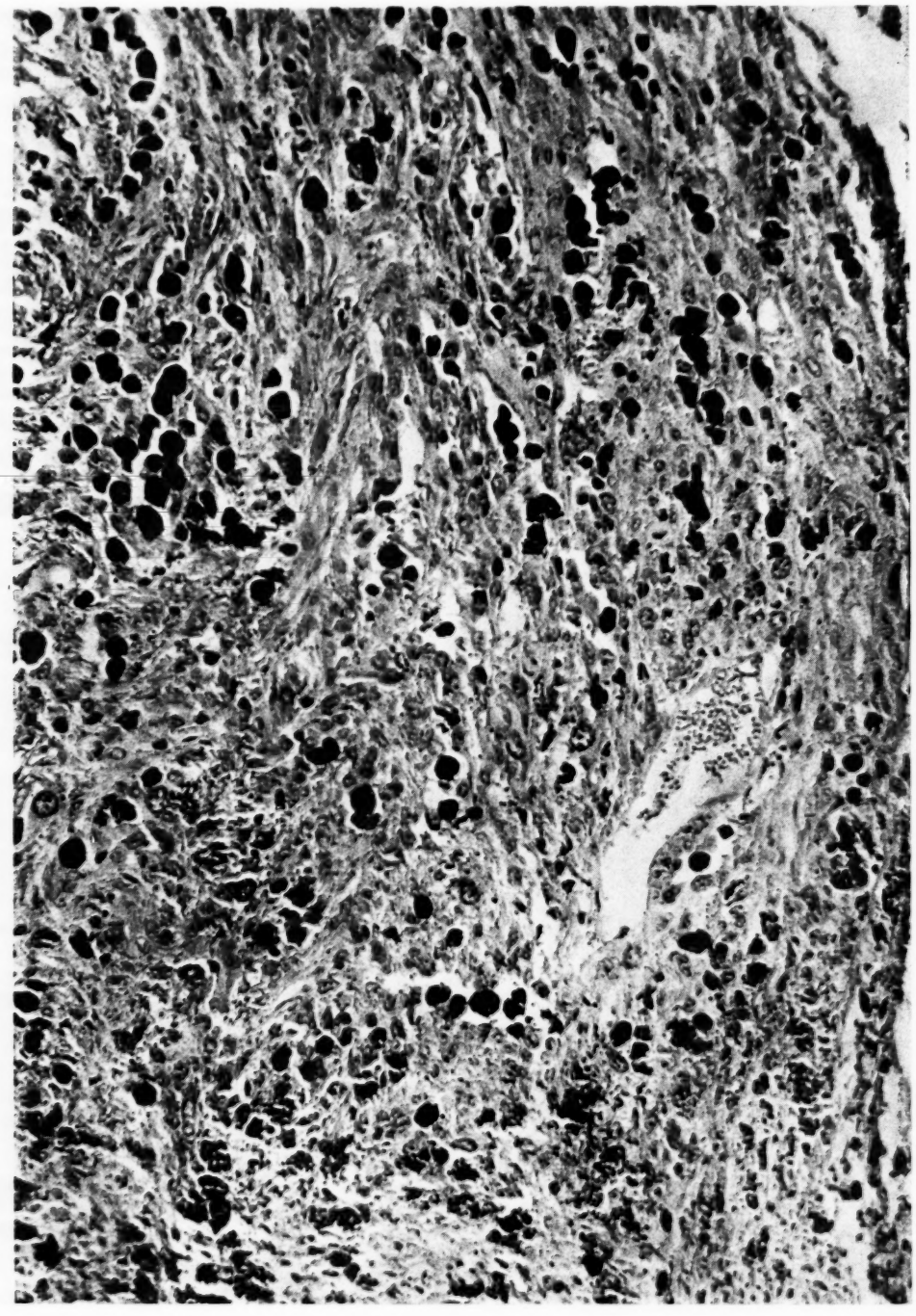

FIG. 2.—A section of the Harding-Passey mouse melanoma taken from the periphery, showing melanotic tumor cells and abundance of phagocytic cells full of melanin. Hexatoxylin and eosin stain. Mag. × 240.

Since there is strong evidence that ovarian activity

or internal ovarian secretion plays an important etiologic role in the development of mammary cancer in animals as well as in the human subject, and that castration leads to the inhibition of prostatic tumors

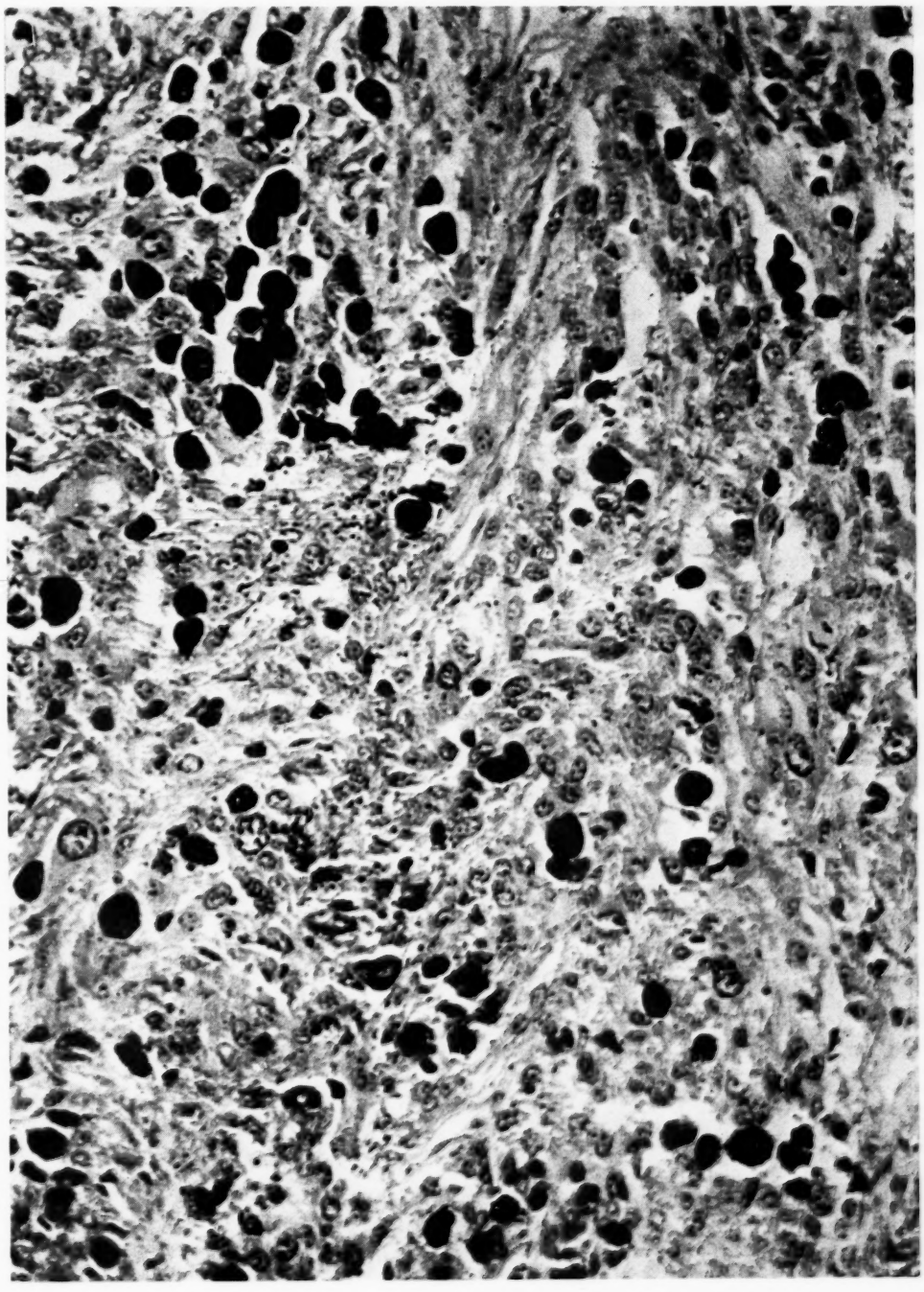

FIG. 3.—Higher power view of Fig. 2 to show large round cells, epithelial types of cell, and mitotic figures. Hematoxylin and eosin stain. Mag. × 325.

in man (5), it appeared of interest to ascertain whether gonadectomy and ovariectomy would influence the susceptibility of animals to transplanted melanoma.

Male and female mice were castrated at 4 to 26 weeks of age. From 1 to 3 months after castration the animals were inoculated with tumor fragments, each weighing about 5 mgm., and the resulting tumors were generally allowed to grow for a period of 4 months. The results are presented in Table II.

The data show clearly that castration did not significantly affect the growth behavior of the melanoma.

In the course of this investigation the effect of castration on established tumors also has been determined. The procedure was the same as that already described except that castration was done when tumor grafts

had grown for 4 to 6 weeks. No effect followed, in either male or female mice.

TABLE I: RESULTS OF TRANSPLANTING HARDING-PASSEY MOUSE MELANOMA INTO SUCKLING MICE OF DIFFERENT AGES

Experiment number	Number of animals	Age at inoculation, days	Percentage of takes	Percentage of regressions
1	6	0.5	100	0
2	8	0.5	75	17
3	5	1	80	0
4	8	1	75	0
5	6	2	67	0
6	8	3	88	14
7	5	3	80	0
8	11	6	73	0
9	7	6	86	17
10	9	7	100	0
11	2	7	100	0
12	8	8	88	0
13	8	9	75	0
14	8	10	100	0
15	8	12	75	17
16	6	12	83	0
17	5	14	100	0
18	6	14	100	0
19	7	16	86	0
20	8	17	88	14
21	10	20	100	0
22	15	21	100	0

TABLE II: INFLUENCE OF CASTRATION AT DIFFERENT AGES UPON SUSCEPTIBILITY TO THE HARDING-PASSEY MOUSE MELANOMA

Experiment number	Number of animals	Sex	Age when castrated, days	Interval between castration and implantation, days	Growth of transplants, per cent
1	10	♂	30	30	100
	10	♀	30	30	80
2	7	♂	30	90	86
	8	♀	30	90	88
3	8	♂	50	30	100
	9	♀	50	30	100
4	8	♂	50	90	75
	8	♀	50	90	88
5	9	♂	180	30	100
	10	♀	180	30	100
6	7	♂	180	90	71
	10	♀	180	90	100

THE INFLUENCE OF HYDROGEN ION CONCENTRATION UPON THE VIABILITY OF THE HARDING-PASSEY MOUSE MELANOMA

Previous studies in this laboratory on the Flexner-Jobling rat carcinoma (20), the Sugiura rat sarcoma I (16), mouse sarcoma 180 (17), and the Rous chicken sarcoma (19) indicated that these tumors showed distinct differences toward the action of hydrogen ions. Knowledge of the relationship between pH and viability of tumor cells is desirable in connection with the cultivation of tumor tissue and chemotherapeutic experiments *in vitro* as well as *in vivo*.

Two sets of buffers were used. One was the standard solutions recommended by Clark, and the other a Locke-Ringer solution previously adjusted colorimetrically to the required pH values with 0.1 *N* and 0.01 *N* solutions of HCl and NaOH. The composition of these solutions has been given elsewhere (19).

About twelve small pieces of tumor, each weighing about 5 mgm., were placed in 25 cc. portions of solution of definite hydrogen ion concentration and salt content. The solutions had previously been sterilized in a steam autoclave at 15 pounds' pressure for 15 minutes. The flasks containing the solutions and tumor fragments were allowed to remain for 24 hours in the refrigerator at a temperature of 4° to 5° C. At the end of this period, the tumor fragments were transplanted into young mice. The results are presented in Table III.

TABLE III: RESULTS OF TRANSPLANTING THE HARDING-PASSEY MOUSE MELANOMA AFTER IMMERSION IN SOLUTIONS OF VARIOUS pH SOLUTIONS AT 4° TO 5° C.

Experiment number	Number of tumor transplants used	Solution	pH	Growth of transplants, per cent
1	10	Clark	4.0	0
	10	"	5.0	30
	10	"	5.8	80
	10	"	7.0	100
2	10	Clark	7.9	100
	10	"	8.9	50
	10	"	10.0	0
3	10	Clark	5.0	10
	10	"	7.0	80
	10	"	8.9	40
4	10	Locke-Ringer	4.0	0
	10	"	7.0	100
	10	"	10.0	0

It is evident that the growth capacity of the mouse melanoma was completely destroyed by immersing in a Clark's buffer solution or in a Locke-Ringer solution at pH 4.0 or 10.0. At pH 5.0, 80 per cent inhibition and at pH 9.0, 55 per cent inhibition occurred. On the other hand, the tumor fragments that had been exposed to a solution at pH 6.0, 7.0, or 8.0 for 24 hours grew normally when implanted into mice. In this respect, the melanoma behaves as the other transplantable mammalian tumors mentioned, but unlike transplantable avian tumors it has a short pH range in which transmissible tumor cells survive.

EFFECT OF DESICCATION UPON THE GROWTH OF THE HARDING-PASSEY MOUSE MELANOMA

It is a well established fact that cancer cells are rendered impotent by the usual desiccation (16, 17). The causative agent of certain infectious tumors of

fowls and of a papilloma of the rabbit are, however, exceptions. The extent of the injurious action of dehydration on melanoma, however, is not known. For that reason the following experiments were made.

Melanomas from 4 to 6 weeks old were cut into small pieces and placed in a cylindrical ampoule for quick freezing. After the tissue was frozen the unit was connected to the pumping system and desiccated for 6 to 12 hours. Dry ice was used to condense the water given off by the tissue (2). The dried fragments were moistened with distilled water or Locke-Ringer solution at pH 7, and then inoculated into young mice. The results were that the transplants that had been dried from the frozen state gave no growths. The conclusion is based upon several sets of experiments.

In the course of the investigation a study was made also, of the resistance of the Harding-Passey mouse melanoma to extreme cold *in vitro*. The results showed that its growth capacity was almost entirely unaffected by sojourn in the frozen state at −70° C. for 7 days prior to transplantation (18).

Nonfiltrability of the Harding-Passey Mouse Melanoma

The production of a mammalian cancer with cell-free extracts or filtrates of cancer tissue has never yet been confirmed, although recently this assertion has been repeated (8, 14, 22).

A weighed portion of fresh melanoma was macerated with sea sand in a mortar, taken up with a quantity of water at pH 7, shaken thoroughly, and kept in a refrigerator at 4° to 5° C. from 6 to 24 hours. This resulted in a 10 to 20 per cent aqueous extract, which was centrifuged and filtered first through a sand and paper-pulp filter (19). A portion of the filtrate was then passed through a Berkefeld filter, grade N. Five-tenths to 1.0 cc. portions of both filtrates were tested, but none elicited a tumor.

Cancer Susceptibility in Relation to Coat Color

Harding and Passey (4) pointed out that many grafts of the melanoma have grown so slowly as to permit the animal a life of 9 months before a tumor of given size is obtained. However, for no apparent reason, some grafts grew very rapidly, and the tumor in this active phase presented new features. To the naked eye it was distinctly paler, varying from a dark grey in one animal to a pale slate color in another. By deliberate selection from the paler tumors these investigators obtained an almost completely colorless growth. Thus from the original heavily pigmented melanoma two distinct strains evolved, one heavily pigmented, and another that is nearly pigment-free to the naked eye. The former maintains its ancestors' slow rate of growth, whereas the latter is much more rapidly growing. Transformation of the heavily pigmented melanoma into nonpigmented melanoma was observed also at the laboratory of the Imperial Cancer Research Fund, London, where the writer was shown such tumors by Dr. J. A. Murray in 1935.

By careful selection from rapidly growing tumors the Harding-Passey melanoma has been propagated through many generations in this laboratory by successive graftings into albino mice of Rockland and Bagg strains, but so far no nonpigmented melanoma has been produced.

It is interesting to note that the original melanoma arose in a mouse that was a uniform milk-chocolate brown.

To ascertain if the color of the host influences the tumor, grafts were inoculated into mice of the following colors: black (C57), dilute brown (dba), brownish-grey (C3H), agouti (New York), and white (Rockland, Bagg, Swiss, Paris). The tumors in these mice were passed successively into animals of a corresponding color, and in this way the tumor has been propagated for several generations in nearly 500 animals. The color of the animal appeared to have no influence upon the susceptibility, the rate of growth, or the depth of pigmentation of the tumor.

SUMMARY

The following conclusions are drawn concerning the Harding-Passey mouse melanoma:

1. Variations in the age of the host did not influence the outcome of transplantation.
2. Suckling mice offered a favorable soil for the continued growth of the melanoma.
3. The growth of transplants in suckling animals neither shortened nor prolonged the lives of animals in comparison with adult animals bearing such neoplasms.
4. Castration in males and females did not significantly affect the growth of the neoplasm.
5. The growth capacity of the melanoma was completely destroyed by immersion in a buffer solution at pH 4 or 10, and at pH 5 or 9 partial inhibition and delayed growth was found. No effect was observable at pH 6, 7, or 8.
6. The viability of the melanoma was completely destroyed by dehydration from the frozen state.
7. The growth was not filtrable.
8. Grafts grew equally well in C57 black, dba, C3H, agouti, and in Rockland, Bagg, Swiss, and Paris albino mice.
9. During the past 15 years the tumor has been

propagated through many generations by successive graftings. So far no nonpigmented melanoma has appeared.

The author wishes to express his gratitude to Dr. George T. Pack for his suggestion of the problem, and to Dr. Fred W. Stewart for interpretation of the histological sections.

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The Relations to Chick Tissues of Tumors Produced by the Yolk Injection Technic

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A successful method for growing large mammalian tumors by injecting a suspension of cells into the yolk of chick embryos has been described by Taylor and his associates (4, 5). Other laboratories have also reported success with the technic (1, 6).

The cancer suspension is injected into the yolk at the 5 day stage of development and when the tumors are examined on the 17th day they appear to be enclosed within the yolk sac. The exact structural relationship between the cancer and the chick tissues is of interest. If the growths were actually inside the layer of endoderm cells lining the yolk sac, they would be separated from the mesoderm and thus from a blood supply. Their large size precludes the possibility that their nutritive and excretory needs can be met by diffusion through the endoderm layer. Also, sections show numerous blood vessels containing chick erythrocytes interspersed through the tumor. The neoplasms evidently establish a mesodermal connection.

The way in which mesodermal attachment occurs was studied by injecting tumor tissue and examining the early stages of its growth. Transplants of the dba mammary carcinoma studied by Taylor (3) were used as a source of tissue. Eggs opened 72 hours after injection and examined under the dissecting microscope showed small aggregations of tumor cells in close proximity to the sinus terminalis. They were well supplied with blood vessels. In sections, the rapidly growing tumor was always found to be external to the endoderm and surrounded by mesoderm, both blood vessels and connective tissue. Photomicrographs of typical sections are shown in Figs. 1 and 2.

Diagrams illustrating stages in the growth of the tumors and the chick tissues are shown in Fig. 3. The establishment of mesodermal connections by tumor cells injected into the yolk depends upon the fact that at the time of injection a large part of the yolk is not enclosed with endoderm. Although the ectoderm in the 5 day egg has grown down and covers most of the yolk, the endoderm covers only the upper half (Fig. 3a). The mesoderm almost covers the endoderm. When the cancer suspension is properly injected into the yolk, the pieces of tissue are scattered through the yolk material and many of them come to rest in close proximity to the ectoderm. The endoderm grows beneath these tissues, thus placing them between ectoderm and endoderm (Fig. 3b). As the mesoderm grows down, it meets the tumor cells, a blood supply is established, and rapid growth takes place.

Because of the need for mesodermal connection, it is clear that implantation of tumor tissue injected into the yolk can occur only during the stages of development in which the endoderm and mesoderm are growing over the yolk. If eggs are injected before this time, the blood supply is not yet well developed. If injected later, the yolk sac has already formed (Fig. 3c), the endoderm encloses most of the yolk, and any injected tumor cells are separated by it from the mesoderm. Since in the 4 day egg the growth of the mesoderm with its accompanying blood supply is already well started, it seemed that injections at this stage might result in suitable implantation and that the extra day for growth would allow larger growths to form. Experiments in which 4 day and 5 day eggs were injected with the same suspension have shown that the tumors from the 4 day injections are significantly larger.

Implantation of neoplastic tissues occurs at the edge of the advancing mesoderm. In 5 day eggs this edge is approximately at the equator of the yolk. However, the growths in the 17 day eggs are almost always found in a small area near the yolk sac umbilicus (Fig. 3d). The implants are apparently carried down with the advancing edge of the mesoderm. The growing regions that cause the rapid extension of the mesoderm over the yolk evidently lie behind the edge (sinus terminalis) which is pushed ahead.

In some 17 day eggs the yolk sac umbilicus is still quite large and in these the individual tumors may be seen to form a ring or a portion of a ring around the albumen. In most eggs, however, the ring has closed almost completely and the individual growths, being brought into close proximity, fuse more or less to form larger aggregates.

In the later stages of growth of the mesoderm over the yolk the edge tends to be folded under. This

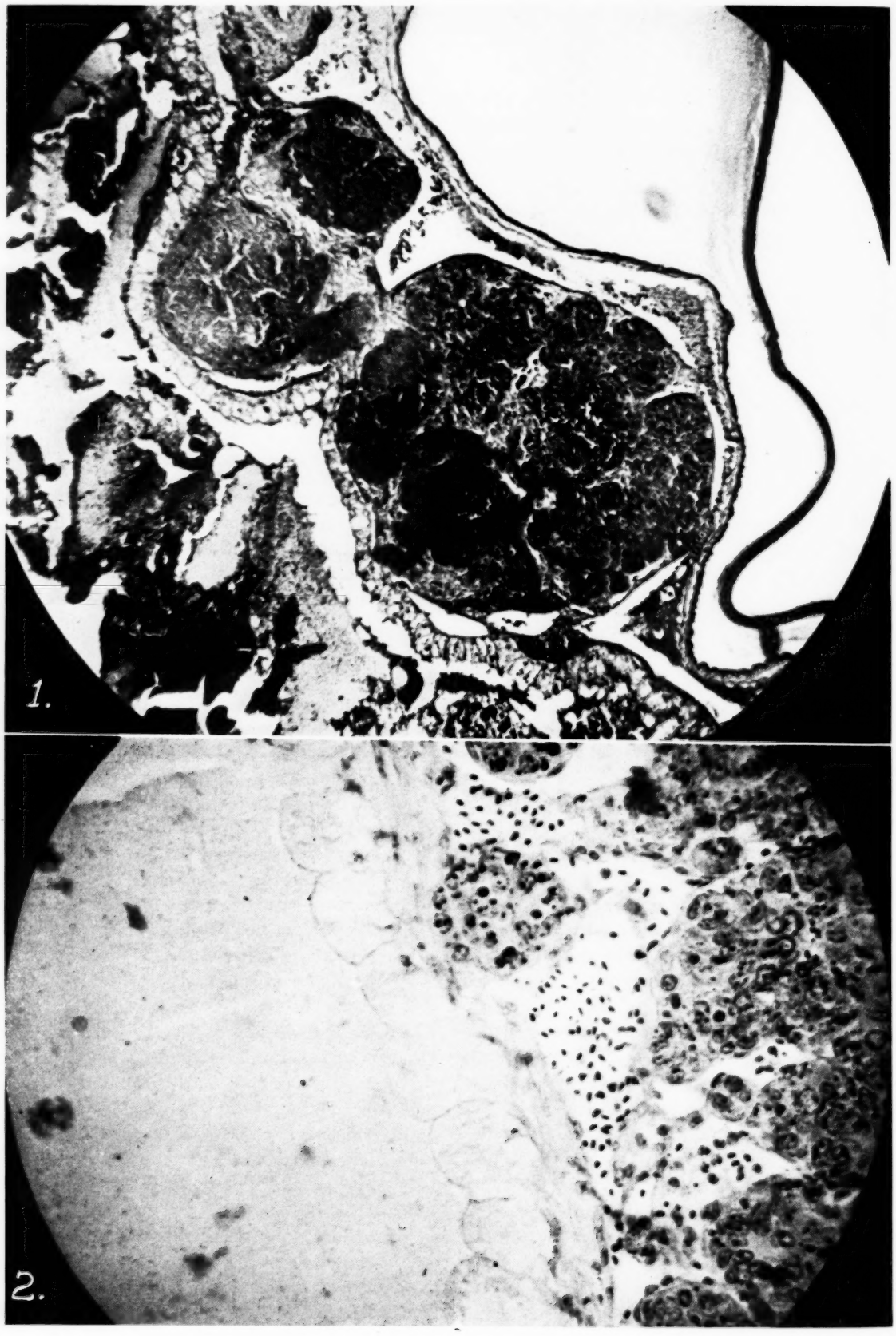

FIG. 1.—Section of tumor 5 days after injection into yolk. Mag. approx. 100 ×.

FIG. 2. Section of tumor in a 17 day egg showing endoderm and connective tissue separating tumor cells from the yolk. Mag. approx. 430 ×.

causes the neoplasms to appear to lie within the yolk sac. Also, the endoderm becomes much folded and extends down into the yolk as pockets in which the cancer tissues lie (Fig. 3d). This, too, causes the tumors to seem to be within the yolk, but sections show that they are still separated from the interior of the yolk sac by the endoderm and that they retain their mesodermal connections and blood supply.

These studies show that the tumors produced by injection into the yolk have essentially the same relation to the chick tissues as those injected by other methods (2). In both cases the neoplasm makes connection with a blood supply by implanting on the mesoderm.

It is evident that only fast growing tumor strains can produce large growths in the developing hen's egg, since 12 to 15 days is the maximum time available. From a given amount of injected tissue the yolk method yields more tumor in 11 to 13 days than can be obtained from a similar injection into the mouse. This agrees with Murphy's observations (2) on the rate of development of neoplasms in the egg. Whether this is due to faster growth or to better implantation has not been definitely determined, though there are some indications that the latter is the important factor.

From the above considerations it is clear that success in growing large tumors by the yolk injection method depends upon the amount of tissue deposited near the ectoderm and remaining outside the endoderm as the latter grows over the yolk. Several technics of injection have been tested with the mouse mammary carcinoma mentioned. The initial method of injecting into the yolk with considerable force has proved to be one of the most important factors in producing large tumors. As an additional measure it has been found useful to rotate the eggs 180° several times after injection. These technics mix the tumor cells with the yolk and disperse them so that many separate tumor particles come to rest against the ectoderm and become established in the mesoderm.

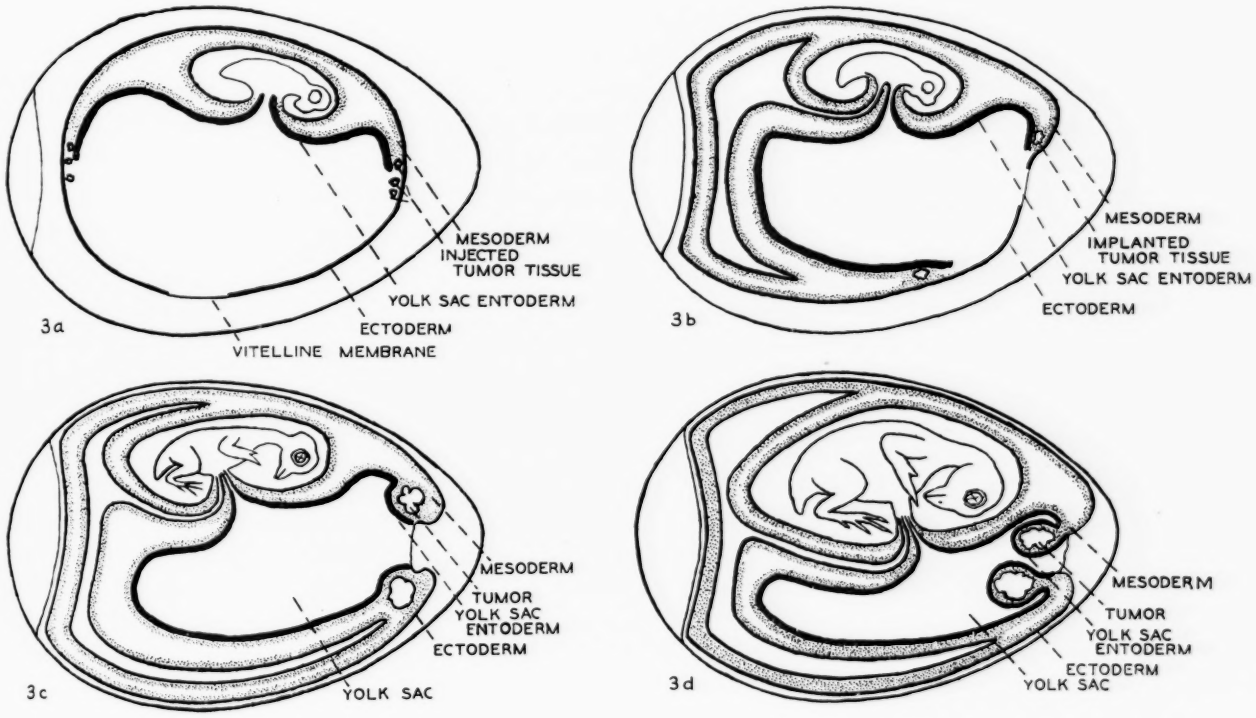

FIG. 3a.—Diagram of a 5 day egg showing position of tumor cells that are becoming established. 3b.—Diagram of a 9 day egg. 3c.—Diagram of a 12 day egg. 3d.—Diagram of a 15 day egg.

SUMMARY

A study of the relationship of egg-grown tumors to the chick tissues shows that implantation and growth depend upon the injected tumor cells making contact with the chick mesoderm. Cells injected into the yolk of the 4 to 5 day egg can make this connection on the edge of the mesoderm since the endoderm does not yet enclose the yolk. The greater the number of implanted tumor cells, the larger is the neoplasm produced. Forcible injection and frequent rotation of the egg aid in giving maximum implantation.

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Is Cancer a Communicable Disease?

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Although the mystery of cancer is as yet far from solved, information recently accumulated from extensive studies on this problem has gradually dissipated a number of misunderstandings and introduced a few new facts that suggest theories quite different from those accepted only a few years ago.

Tumors are probably as common in frogs, fish, flies, chickens, mice, rats, rabbits, dogs, or horses, as in man. In animals, as in man, cancer is usually a disease of older age, and is only occasionally observed in young subjects. Tumors may arise in almost any part of the body; some grow rapidly and kill their hosts in a comparatively short time; many others grow slowly; still others may persist for years without much injury to their hosts.

I

The Inoculation of Tumors in Animals

Tumors that develop in animals can be excised and reinoculated into the same host, or transplanted from one animal to another. Although tumors can usually be successfully transplanted only within the same species, or even race, as from mouse to mouse, rat to rat, rabbit to rabbit, etc., transplantation between animals of different species is also possible. By implanting into the anterior chamber of the eye instead of subcutaneously tumors can be successfully transferred from one race, species, or even order, of animals to another, for instance from mice to rabbits, rabbits to guinea pigs, etc.

It was first thought that tumors could be transferred only by large particles of undamaged cells, because the first new growths found in rats and mice could not be transmitted by cell-free filtrates. Later it was discovered that malignant neoplasms in chickens, and certain tumors in frogs, rabbits, and dogs are often readily transmissible by cell-free filtrates or by tumor tissue that has been either desiccated or stored in glycerine. Recent studies indicate that mammary cancer in mice also can be transmitted by cell-free filtrates if certain experimental conditions are met.

Acquired Specific Immunity to the Implantation of Tumors

Successful implantation of a malignant growth into a susceptible host is not necessarily fatal for the animal. In certain instances the implanted tumor grows only temporarily and eventually disappears, and even one that is highly malignant for certain races or inbred lines of animals may occasionally regress.

It has long been observed that animals that have exhibited complete, spontaneous regression of successfully implanted tumors usually resist reinoculation with the same neoplasm. On the basis of this observation a method of active immunization was devised by Besredka and the writer a few years ago (11, 12). Susceptible animals were inoculated intradermally with a very small amount of finely ground tumor-cell suspension; under proper control of dosage the resulting intradermal tumors regressed in an appreciable number of instances and animals in which this occurred were found immune to reinoculation of the same neoplasm by any route. This procedure has been applied successfully in mice (11, 12), rabbits (16, 13), and chickens (14). It has also been observed in recent experiments that temporary growth of an intradermally implanted tumor, followed by its spontaneous and complete regression, confers immunity on the host no matter what the origin of the tumor or its genetic relation to the inoculated host (38). This immunity is directed specifically against the tumor used for the immunizing inoculation. Although it is substantial (40) and durable it cannot be transferred passively from one animal to another, even by the injection of large amounts of serum or of ground organs from immunized animals (13, 14).

The immunity acquired by spontaneous recovery from tumors presents a complete analogy with that observed in diseases caused by various microorganisms and viruses. Many centuries ago men observed that persons who had recovered from certain infectious diseases, such as smallpox or measles, usually possessed a lasting immunity. This is most striking in certain virus infections although it exists in other communicable diseases also. Thus observation that susceptible animals can be successfully immunized against the experimental implantation of neoplasms is of considerable significance. At the present time, however, no more than theoretical importance can be attached to these experiments since no proof has yet been furnished that intradermal immunization may also

prevent the development of tumors naturally occurring in animals.[1]

The "Spontaneous" Development of Tumors

Although malignant growths develop frequently in various animals, in most instances no evidence has been found to suggest that a tumor-bearing animal can transmit the disease to another. Whereas neoplasms can be transplanted artificially from one animal to another, no similar transfer seems to occur in nature; for a handful of tumors only, such as the rabbit myxoma or rabbit papilloma, were data at hand to indicate transfer under natural conditions, but since no such evidence could be found for the great majority, it seemed logical to assume that each of these tumors is a separate entity, arising *de novo* in the afflicted animal without any causal connection with similar neoplasms in other animals. Hence, with few exceptions, each tumor that developed in a normal, untreated animal was considered to be a "spontaneous" new growth.

The "Cancer Families" in Mice

During early experiments on the transplantation of tumors several investigators observed that spontaneous neoplasms could be transplanted without difficulty from one sibling to another, but that such transplantations only rarely succeeded in other animals. By successive transplantations a tumor would only gradually acquire sufficient virulence to grow indiscriminately in various animals of the same species, if its virulence were enhanced at all. Since the number of takes in "market" mice, *i.e.,* in animals of promiscuous origin, was wholly irregular, several investigators began inbreeding mice to obtain a sufficient number of siblings or sibling-like animals equally susceptible to implantation. Thus female mice have been mated in each generation to their brothers only, a process that eventually resulted in a high degree of genetic uniformity in all animals of a particular inbred line. For practical purposes these animals were as alike as identical twins, and they proved to be equally susceptible or equally resistant to the implantation of certain tumors.

Several lines of mice have been thus established (74—77), each designated by a letter symbol such as A, C, C3H, and so on. It was soon observed that the animals of some of these lines developed spontaneous tumors when allowed to reach older age, whereas mice of other lines remained free. The comparatively short life-span of these rodents seemed to afford a good opportunity for experimental study of the inheritance of these naturally occurring neoplasms, and interest switched from transplanted to spontaneous tumors.

Mice of some of these lines have been found practically free from neoplasms, no more than 2 or 4 per cent developing them; the C, C57 black, CBA, and other similar lines are therefore called low-tumor lines. On the other hand, certain inbred lines like the A, or the C3H, show a remarkably high incidence of spontaneous tumors in each generation. These are called high-tumor lines.

The Influence of Sex and Age on the Development of Spontaneous Tumors

A striking influence of sex and age on the development of spontaneous tumors was immediately observed in these strains. Thus practically all females of the C3H line have mammary carcinomas before they reach 1 year of age. Females that have had litters develop mammary tumors earlier (5) than virgin females. Male animals of this line develop neoplasms of the liver or lungs at an average age of 15 months (1); many of them, however, die without tumors, or before they reach the comparatively late tumor age.

The females of the A line differ from those of the C3H line, since there is a striking difference in the incidence of mammary cancers between the breeding and virgin females (74, 17, 19). Practically all breeding females of the A line develop mammary carcinomas at the average age of 12 months; on the other hand, virgin females of this line, with few exceptions, remain free from mammary tumors. They develop, however, lung carcinomas (17) upon reaching the average age of 17 months. Males of this line also develop lung carcinomas at approximately 15 months of age (17).

Males of the high-tumor lines do not normally develop mammary tumors. When castrated early in life, however, and engrafted with ovaries (58), or when injected with estrogenic hormones (48), they have mammary tumors about as often as virgin females of the same line. It should be pointed out, though, that animals of low-tumor lines do not develop mammary cancers from overstimulation with estrogenic hormones.

Thus it seems that hormonal stimuli, coupled with changes that are associated with older age, have only an intermediary influence on the development of certain spontaneous tumors, such as mammary cancer, in mice. It may be that in certain animals old age, together with an alteration in the hormonal balance, may activate a pre-existing but hitherto latent tumor factor, and thus be only indirectly responsible for

[1] For bibliography on transplantation of tumors and experimental tumor immunity see (39), and (92).

the development of at least some of the "spontaneous" neoplasms.

Mammary Cancer in Successive Generations under Promiscuous Breeding

The studies on the development of spontaneous tumors in successive generations of "cancer families" of mice have been carried out on genetically uniform animals of pure inbred lines. Clearly the breeding conditions are artificial, and cannot be compared with those occurring in nature. A similar development of spontaneous tumors in members of successive generations of mice has been observed also, however, under the usual conditions of promiscuous breeding. Thus Dobrovolskaïa-Zavadskaïa (29) obtained 5 female mice of unknown origin with mammary carcinomas. Two of these were mated to males that had been born to cancerous mothers, and 3 to males of unknown origin. There was a striking incidence of cancer in the resulting progeny. All females of the first generation that lived over 6 months developed mammary tumors, and of the 84 females in the second generation 56, *i.e.,* 67 per cent, died with mammary carcinomas.

In other and similar experiments (28) a cancerous female mouse of unknown origin mated successively to 3 different males had 7 cancerous and 5 noncancerous daughters. One of these cancerous daughters mated to her own brother had noncancerous offspring, but her 2 granddaughters developed mammary tumors.

Transmission of Cancer Through Mother's Milk

For a number of years elaborate genetic theories were advanced to explain the origin of spontaneous tumors, and especially mammary cancer, in mice. A few years ago, however, it became clear that chromosomal factors could not be held exclusively responsible for their development. It was observed by members of the Roscoe B. Jackson Memorial Laboratory (73) that females of a high-tumor line mated to a male of a low-tumor line were able to transmit the tumor factor to their offspring in a high percentage of cases, whereas males of the same high-tumor line mated to females of the low-tumor line had offspring in which few, if any, tumors appeared. It was concluded that some maternal or extrachromosomal influence responsible for the development of spontaneous tumors had been transmitted from the mothers to their progeny.

The Prevention of Breast Cancer by Foster Nursing

The maternal influence could be transferred by cytoplasmic inheritance, during intrauterine development, or by way of the mother's milk. Suspecting milk transmission, Bittner (18), early in 1934, removed young mice born to high-tumor A line females from their potentially cancerous mothers before they were 24 hours old, and transferred them for foster nursing to lactating females of the low-tumor CBA and C57 black lines. The results were striking (19). Whereas 96 per cent of 376 breeding females of the A line developed spontaneous mammary cancers at the average age of 10 months, no more than 8 per cent of the 127 fostered females did so; 92 per cent of these died at the average age of 17.7 months without tumors.

These dramatic observations have been repeated and confirmed by other investigators, and with various lines of mice. Andervont (2—6) observed that foster nursing of females of the high-tumor C3H line by low-tumor females of the C, Y, or I lines reduced the incidence from 100 to 20 per cent. The appearance of spontaneous mammary cancer could be entirely prevented in C3H females by removing these animals at full term directly from their mothers' uteri and foster nursing them on females of the low-tumor C57 black line. It has been observed that removing mice from their mothers 24 hours or later after birth had little or no effect on the future development of tumors in these animals; in order to prevent the appearance of neoplasms in such mice they had to be removed immediately after birth.

Thus it was definitely established that the development of breast cancer can be prevented in mice of high-tumor lines by isolating newly born animals from their potentially cancerous mothers and transferring them for nursing to lactating females of low-tumor lines.

In recent experiments Bittner (22) removed C3H females on the day of birth from their potentially cancerous mothers, and transferred them to females of the low-tumor C57 black line. Through 5 successive generations only 1 per cent of 165 descendants of these fostered mice developed tumors; the remainder died at the average age of 16.9 months without neoplasms. Of the 214 control C3H females 97 per cent developed mammary cancers at the average age of 8.9 months.

Hence a high-tumor line changed into a low-tumor line after foster nursing of females from one single generation of a high-tumor line by lactating females of a low-tumor line.

Transmission of Mammary Cancer to Mice of Low-Tumor Lines

A logical sequel was to determine whether mice of a low-tumor line would develop mammary cancer when nursed by females of a high-tumor line.

It was soon observed by Andervont (2) that mice of the low-tumor C line developed mammary cancers in 64 per cent of cases when nursed by females of the high-tumor C3H line. None of the control females of the C line nursed by their own mothers developed tumors. In other experiments (4) the incidence of mammary cancer in low-tumor hybrid females, derived from mating of C57 black and I lines, was increased from 0 to 80 per cent by transferring the newly born animals to C3H females. The tendency to develop mammary cancer, once acquired, could also be transmitted through successive generations. Thus when acquired by females of the low-tumor C line after foster nursing by females of the high-tumor C3H line it was transmitted to the second and third generations (3); a low-tumor line changed into a high-tumor line after foster nursing of females of one generation of a low-tumor line by females of a high-tumor line.

The tumor factors carried by various high-tumor lines are probably not identical, since they may have a different affinity for animals of various inbred lines (4). Conversely, animals of a low-tumor line may prove susceptible to a tumor factor carried by one high-tumor line, but may at the same time have a low susceptibility to a tumor factor carried by another high-tumor line.

Importance of Susceptibility to Cancer Compared with That to Various Infectious Diseases

According to the generally accepted theory (19, 20), three factors are responsible for the origin of mammary cancer in mice: (a) the transmissible milk "influence," which may, however, according to Bittner (20), arise *de novo* within the individual; (b) inherited susceptibility; and (c) hormonal stimulus.

This theory does not sufficiently stress the importance of a transmissible causative agent in the origin of mammary cancer in mice; on the other hand, the importance of inherited susceptibility as well as the influence of hormonal stimuli seems to be overemphasized, and may be misinterpreted. Thus few bacteriologists, if any, would state that anthrax, for instance, is caused by two factors: (a) the transmissible anthrax bacillus, and (b) inherited susceptibility. The anthrax bacillus only would be mentioned, since it goes without saying that this microorganism can act only on a susceptible host. Susceptibility is obviously fundamental in the acquisition of *any* communicable disease, since pathogenic microorganisms or viruses can act only on susceptible cells within susceptible hosts. Some microorganisms have a wide range of action; others are limited in their pathogenic activity to susceptible races, families, or even individuals. These microorganisms are nevertheless considered the true causative agents of the resulting diseases. Thus Algerian sheep, in contrast to European, are relatively insusceptible to anthrax; field mice are relatively resistant to streptococci or pneumococci, whereas albino mice are very susceptible to these organisms (33). Webster (87) selected susceptible and insusceptible parents for breeding and eventually obtained two lines of mice, one showing 95 per cent mortality and the other only 5 per cent under the same conditions of exposure to *B. enteritidis*.

Hormonal stimuli may also distinctly influence the susceptibility of the host to certain communicable diseases. Thus a higher susceptibility of males, as compared with females, to many infectious diseases has long been observed in human and animal pathology (33). Some diseases, such as ringworm of the scalp (*Tinea tonsurans*), occur in childhood but rather seldom in sexually mature individuals (69).

The susceptibility of the host, fundamentally inherited, but also strongly influenced by hormonal stimuli, age, and various environmental factors, seems, at least in principle, no more important in the origin of cancer than it is in the acquisition of diseases caused by various microorganisms and viruses.

Mammary Cancer in Mice—A Communicable Disease

Upon reviewing the problem dispassionately it is difficult to avoid the conclusion that mammary cancer in mice is a communicable disease transmitted through the milk of nursing animals from one generation to another. Animals that transmit the tumor factor and are responsible for the dissemination of the disease do not themselves display any apparent symptoms at the time of transmission; they develop tumors, if at all, later in life. They are carriers of a temporarily latent tumor factor, which is probably a virus (20): they have an asymptomatic neoplastic disease. Young animals that acquire the tumor factor by suckling milk from their potentially cancerous mothers do not have mammary cancer until they reach approximately 1 year of age, and some die without tumors. In the meantime, however, they become carriers, and before displaying any symptoms may again transmit the disease to the next succeeding generation.

Although these observations refer thus far to mammary cancer in mice, there is no reason to believe that this form of tumor has an etiology essentially different from that of other neoplasms.

The Law of Obligate Communicability Probable Also for Tumors?

The generally prevailing supposition that cancer is a "spontaneous" disease, caused by various internal

and external factors, is not unlike the ancient conception of "spontaneous tuberculous degeneration" advocated by Pidoux (82) and other believers in "morbid diathesis." At the time of Villemin tuberculosis was thought to be "a common result of a quantity of diverse external and internal causes" (82), and it is worth emphasis that as late as 1885 typhoid fever and diphtheria were believed to originate *de novo* in filthy surroundings (66). It took many years to demonstrate that the law of obligate communicability still prevails for all infectious diseases. The same may be true for cancer. The conception of the spontaneity of at least certain of its forms seems untenable in view of the experimental evidence suggesting that what was thought to be "spontaneous" mammary cancer in mice is a disease communicable from generation to generation, and that its appearance can be effectively prevented by isolating the potentially cancerous mothers from their newly born progeny.

It is true that while mammary carcinoma in mice has been found to be communicable from one animal to another, no experimental evidence has been obtained as yet for a similar natural transmission of other tumors, such as various sarcomas, etc. It is possible to assume, however, that the mammary carcinoma of mice does not represent a form of cancer fundamentally different from other neoplasms. If this assumption is correct, the law of obligate communicability may sooner or later be established for other tumors also.

Thus far milk has been recognized as the intermediary factor transmitting the disease from generation to generation. Other means of transmission, however, such as ovarian transfer, should also be considered. Cancer is not limited to mammals, and it seems reasonable to assume that tumors can be transmitted by means other than milk, not unlike various microorganisms that are transmitted from one generation to another (81, 71, 60, 32).

Occasionally the same animal may simultaneously carry several different tumor factors, such as lung or bone tumor factors, and a mammary carcinoma agent. Although the appearance of mammary cancer may be prevented in the next generation by isolating the potentially cancerous mothers from their newly born progeny, this precaution will not necessarily eliminate the bone or lung tumors, since these may have ways of transmission other than milk.

II

Let us now consider the few available experimental data and observations referring to the transmission of human cancer: (a) in the same person, (b) from one person to another, and (c) from one generation to another.

Autoinoculation of Human Cancer

Accidental contact transplantations of tumors in the same patient, as from one lip to another, from tongue to gum, from one vocal cord to another, from breast to the adjacent chest skin, etc., have long been observed by various authors (10, 23, 30, 50). Accidental transplantations of tumors during laparotomy, abdominal paracentesis, or débridement of a tumor through the vagina, have been described by numerous surgeons (50). An accidental transplantation of an adenocarcinoma from the breast to the skin of the thigh in a 43 year old white female was observed by Spies and his colleagues (72); 2 months after the plastic transplantation of Reverdin grafts from the patient's right thigh to the granulating wound surface of the left chest wall a nodule developed in the skin of the right thigh where the autografts had been excised. The microscopic report on this nodule (Ewing) was "a very cellular infiltrating carcinoma of mammary gland type." A similar case of accidental transplantation of a breast tumor to the skin of the arm was observed by Hubard (44).

Cornil reported (26) that an "anonymous surgeon" transplanted in 1887 a breast sarcoma and a breast carcinoma, each into the patient from whom it had been excised. The implants grew after incubation periods of 2 months and a few weeks, respectively. The sarcomatous implant was excised and found to be histologically identical with the original breast sarcoma. The carcinomatous implant grew, but was not excised. Similar cases of successful, intentional autotransplantation of human cancer were reported by Delbet (cited by Kurtzahn) and Hahn (41).

More recently de Martel (54) transplanted carcinoma of the breast (3 patients) and carcinoma of the bowel (1 patient) under the skin of the abdomen, and each into the patient in whom it had originated. A latent period of approximately 5 months was observed in all 4 instances; after this had elapsed the implants began to grow very rapidly. They were destroyed in all patients without difficulty by x-rays.

On two occasions Besredka and Gross (15) implanted intradermally a carcinoma excised from the breast into the patient in whom it had originated; both already had metastases at the time. The intradermal implants grew in both cases after an incubation period varying from 6 weeks to several months; they were later removed and proved to be microscopically identical with the original tumors used for inoculation.

A melanosarcomatous metastasis was excised and immediately reinoculated intradermally in a 53 year old woman by Gross, Zinninger, and Ulin (37). The implant grew after an incubation period of 10 weeks

and proved to be microscopically identical with the original tumor used for inoculation.

Transplantation of Cancer from Man to Man

A few dramatic observations of accidental transplantation of malignant tumors from man to man have been recorded by competent observers. According to Tross (79) a man developed a carcinoma of the glans penis presenting a structure histologically identical with the cervical carcinoma from which his wife suffered. A patient reported by Martin (55) suffered from a recurrent epithelioma of the external ear and neck. This patient "was most free from pain when resting his head on the breast of his wife, the stained dressing often being in contact with her bare skin." A few weeks after the patient's death Martin was called to attend the widow, and found her suffering from an epithelioma of the chest wall, just above the left breast, in the exact place where her husband's head most often had lain. A similar case of probable transplantation of cancer from a woman's breast to her husband's gum was reported by Peyriche (63). Balacesco and Tovaru (7) reported the case of a young woman who had a small nodule in her right breast. The woman delivered a baby and at this time the nodule in her breast ulcerated. Nevertheless, she nursed her baby. After 11 months the child developed a tumor on its lower lip. Both mother and child were at this time operated upon, and the breast tumor as well as the lip tumor were excised. The mother proved to have an adenocarcinoma of the breast, the child had a spindle cell sarcoma of the lip. In spite of the microscopic difference in appearance of these tumors, there is a strong possibility that the tumor was transmitted from mother to child by contact inoculation.

Weber and his associates (86) reported the case of a melanosarcoma most probably transmitted from a 27 year old mother to her child. The full term child had been delivered by cesarean section in apparently good health 3 months before the woman died of generalized melanosarcoma; the physician (E. Holland) who delivered the child remarked that "the lower uterine segment of the mother was during the Caesarean section occupied by a huge black placenta proved to be infiltrated with masses of tumor" (86). He thought it probable that tumor cells might have been carried in the placental blood stream from the mother to the liver of the fetus. The child died after 8 months from generalized melanosarcoma with large tumors in the liver.

Lecène and Lacassagne (49), and later Katz (45), described the dramatic case of a fatal accidental inoculation of human cancer into the hand of a medical student, Henri Vadon. The unfortunate Vadon aspirated serum from a wound following radical mastectomy in a woman suffering from an adenocarcinoma of the breast. The syringe slipped, however, and while falling accidentally punctured his left hand, injecting some of the fluid deep into the tissues of the palm. This happened on the 13th of February, 1923, in a ward of the Cochon hospital in Paris. In February 1925, *i.e.,* two years later, an irregular induration appeared at the site, with a distinct prolongation toward the old scar remaining after the puncture, and tuberculous synovitis was suspected. The induration increased slowly in size. It was removed in August, 1925, and sent for histological examination. This revealed a fusiform sarcoma. The tumor recurred in the scar and metastases rapidly appeared under the skin of the internal surface of the arm. On September 23rd the whole arm was disarticulated by Lecène; 2 months later, however, metastases appeared above the clavicle and in the neck. Vadon died on December 12th, 1926, one year after the metastases appeared. Thus he inoculated himself with a mammary epithelioma, and developed a fusiform sarcoma at the site of inoculation. There had been no tumors in Vadon's family for 3 preceding generations on his paternal side; on the maternal side, one grandfather died of a suspected tumor of the kidney.

It should be emphasized that striking histological changes occurring in tumors in the course of experimental transplantation from one animal to another have been repeatedly observed by numerous competent observers. Thus during serial transplantation of a carcinoma a sarcoma may eventually develop. Such transformation may occur more or less rapidly following successive transfer of the neoplasm. Whether the transformation is genuine, *i.e.,* whether the carcinoma actually changes into a sarcoma, or whether the transformation is due to a sarcomatous change in the stroma that gradually overgrows the carcinoma is of considerable theoretical importance, and has been fully discussed by Woglom in his review of experimental cancer (91). From a practical point of view, however, the fact remains unchallenged that following transplantation of a carcinoma from one subject to another, a sarcoma may develop. Thus it is possible to assume that a similar histological transformation may occur also in human neoplasms accidentally transplanted from one person to another. This would perhaps explain the case of Vadon (45, 49), and also that described by Balacesco and Tovaru (7).

Transplantation of Cancer from Man to Animals

Human sarcoma or carcinoma can be transplanted without difficulty into the anterior chamber of the eye of various species of animals, such as rabbits,

guinea pigs, etc. After an incubation period varying from 3 weeks to 4 months the implanted tumors begin to grow rapidly, and can then be transferred by serial passage in the new hosts. Thus a human fibrosarcoma could be transferred for 14 consecutive passages in guinea pigs (36). The incubation period may rapidly diminish during successive transfers so that eventually tumors will grow within 7 days following implantation. After several ocular transfers the tumor may be successfully inoculated by other routes, for instance into the testicle.

Thus examples of either accidental or intentional inoculation of human cancer in the same subject, or of unintentional transplantation of a tumor from one person to another, are comparatively rare; not all such observations have been published. Yet the available experimental data, however scarce, suggest that the analogy between neoplasms in animals and in man is not limited to the microscopic appearance of tumors and to their clinical evolution. Like tumors observed in animals, human cancer is unquestionably inoculable in the same person, or from man to various species of animals. Although no sufficient experimental data are as yet available, it seems logical to assume that under certain conditions human cancer can be transplanted also from one human subject to another.

Appearance of Cancer in Families and Successive Generations in Man

It has been observed repeatedly that tumors may develop in several members of the same family. Although cancer is a comparatively common disease, and the accidental occurrence of tumors in more than one member of the same family must be considered, a number of "cancer families" has been observed with such a striking incidence of tumors (62, 85) that mere chance can reasonably be excluded. Broca observed breast cancer in a woman whose 4 daughters had cancerous tumors (24); of the 16 grandchildren, 8 died with neoplasms. Finney (31) reported a family in which the mother, 4 daughters, and 3 nieces had all been operated upon for cancer at the Mayo Clinic; all but one had cancer of the breast. Graham's (35) patient had cancer of the rectum; her 3 daughters and 1 son all died of cancer of the rectum. Handley (42) reported 4 sisters with breast cancer; their mother and grandmother also died of tumors.

Körbler (46) reported that by questioning 426 patients treated for tumors he was able to find 4 cancer families. In one case both parents and their 3 children died of cancer. In another case a mother, 4 daughters, and a son died of tumors. Most interesting was the third case, where 5 of 7 children of apparently healthy parents developed tumors. One of the tumorous daughters had 3 daughters, all of whom developed cancer of the breast. Two granddaughters of this patient also developed breast carcinomas. Thus cancer appeared in members of 3 successive generations of this family. Körbler cited another in which both parents and their 3 children developed carcinoma. Wood and Darling (93) reported a family in which bilateral carcinoma of the breast, or other tumors, developed in numerous members of 4 successive generations. Three sisters of the third generation all developed cancer of the breast.

Williams (90) treated a woman with uterine cancer whose maternal grandmother, mother, and mother's sister all died of cancer of the uterus; two sisters of the patient also had cancer of the uterus. Manson (53) treated a 27 year old woman who developed a fatal sarcoma on the left side of her neck; two of her 3 sons died of identical sarcomas at the same site. Power (65) reported a cancer family in which the father, 2 sons, and 6 daughters all died of cancer.

Silcock (70) reported melanosarcoma of the eyeball in 3 successive generations. Another highly malignant tumor of the eye that affects infants, the retinoblastoma, is a very rare disease, 1 case being observed in 34,000 births; in many families, however, half of all the children are afflicted (89). "Only recently," stated Weller (89), "has it been appreciated that the disease may exhibit also a vertical familial distribution appearing in successive generations, or in collateral lines." Thirty families giving evidence of the appearance of retinoblastoma in successive generations have been collected (88, 89).

Instances of the concurrent development of cancer of the stomach in brothers (59) or twins (57), or of the appearance of tumors of the breast (25) or ovaries (80) in twin sisters, or of the concurrent development of osteogenic sarcoma in brother and sisters (67), or sisters (64), have been compiled (51). Numerous statistical studies on cancer families and on hereditary factors in patients suffering from tumors have been conducted (8, 27, 43, 46, 51, 52, 56, 61, 84, 85, 88), leaving little doubt that cancer is definitely more frequent in the families of cancer patients than in the average population. Although most cancer patients seem to represent sporadic cases, the number of these cancer families is impressive.

Studies on cancer families have been limited to 2 or 3 successive generations with few exceptions, and it has been established that the disease can follow members of certain families for the number of generations observed. There is no reason to doubt that a similarly high incidence of tumors may occur in both preceding and succeeding generations. Other families,

on the contrary, are essentially exempt, practically no cancer having appeared in several generations.

Analysis of the available data does not suggest that any hereditary mendelian factor, either recessive or dominant, determines the development of cancer in animals or in man. The late Prof. A. S. Warthin, in a statement to his class in pathology in 1929, summarized the work completed to that time thus: "Heredity may show itself in some cases as recessive, in others as dominant, and in still others in a hit-or-miss fashion" (19). The genetic explanation of cancer thus proved clearly inadequate. On the other hand, recent studies have demonstrated that mammary cancer in mice can be either prevented, or acquired in early infancy, through the ingestion of milk from tumor-free but potentially cancerous mothers. Although it is true that inherited susceptibility is essential for the development of tumors, cancer does not differ in this respect from other communicable diseases. Susceptibility is indeed of paramount importance in the development of *all* infectious diseases; the infecting microorganism or virus is, however, ultimately responsible for the disease.

III

Theoretical Implications of the New Approach to the Cancer Problem

The observations discussed above suggest the possible existence of external factors transmitted from generation to generation, and responsible for the appearance of tumors in animals and in man. Transmission of various infectious diseases from one generation to another has been observed (81, 71, 60, 32), and cancer would not be the first disease known to be communicable from one member to another in 2 successive generations. Although it can be artificially or accidentally transplanted from one subject to another within the same generation, a natural or spontaneous communicability of cancer seems to take place between individuals of successive generations. This may be true at least for certain forms of the disease, such as the mammary carcinoma of mice. This fact contributed to the difficulty in understanding the nature of cancer as a communicable disease, especially since a long interval of time may intervene between the development of the initially observed and the transmitted neoplasm.

Thus individual instances of the development of tumors should be viewed in their proper perspective, *i.e.,* as single links only in a continuous chain of a disease that is being transmitted from one generation to another. In order to appreciate the communicability of tumors, the time factor should be disregarded; their communicability may then be compared in principle with that of various infections, except that diseases thus far recognized as being contagious spread, as a rule, among members of the same generation. One is almost tempted to admit the possibility of an "epidemic in the fourth dimension." Human life may well be too short to permit any one investigator to grasp the concept of the communicability of cancer in man; the time factor may prove to be one of the most formidable obstacles in further research on the epidemiology of tumors.

The Vertical Epidemic of Cancer

In a normal or "horizontal" epidemic, that of smallpox or common cold, for instance, the chain of infection spreads among individuals of the same generation, and the whole picture of a communicable disease, as such, is clearly discernible to anyone; thus several hundred consecutive cases of transmission of the disease from one individual to another may occur within a comparatively short time, and certainly within the lifetime of the human observer. In a "vertical" epidemic of tumors, however, one human observer can see but a very few scattered links in the chain of communication. The comparatively short life span of the investigator does not permit him to follow the spread of the disease for more than a few consecutive transmissions, and the communicable nature of the disease may therefore entirely escape the individual observer. One single physician, driving his car, could visit during one day a dozen patients who contracted smallpox, or common cold, one from another. A human observer, however, could visit several persons who had contracted breast tumors one from the other only if he were able to travel in the fourth dimension, *i.e.,* if he were able to travel in both space and time.

Another reason apparently responsible for the difficulty in understanding the problem of cancer is the fact that most of those who spread the disease are latent carriers; they do not themselves display any apparent symptoms, but many of them develop tumors upon reaching old age, or under the influence of certain hormonal, or as yet obscure, stimuli. Some die before they reach the tumor age, and some remain free from tumors even though they do reach old age. They transmit the disease, however, not unlike apparently healthy carriers of various infectious diseases in animals and in man. The carriers of typhus rickettsias, for instance, may remain asymptomatic yet spread the disease, and may, for obscure reasons, themselves develop clinical symptoms later in life (Nicolle).

It may well be that at least certain forms of malignant tumors are caused by an invisible virus, whose existence depends upon close association with the cells of a living host. Such a tumor virus would be highly adapted to certain hosts, and probably also to

certain cells within the host, a fact repeatedly observed in various virus diseases. As a rule the tumor virus would be frugal and moderate in its requirements and would not, apparently, injure the host; it would behave like a really "efficient parasite (78)." For obscure reasons the pathogenicity of this virus may sometimes change during the later life of the host, and a tumor would then develop. In most instances the fate of the host would thus be sealed, the neoplasm growing progressively and eventually killing him. Occasionally, however, a tumor may grow only temporarily and then spontaneously regress. Cases of spontaneous regression of human cancer, though unusual, have been repeatedly recorded by competent observers (34, 68), suggesting that similar regression of small and unrecognized tumors may perhaps occur more often than is generally assumed. In any event the survival of the virus would have been secured by transmission to the next generation of the host.

There may exist a group of fundamentally similar but individually distinct tumor viruses causing various neoplasms, not unlike the array of rickettsial microorganisms responsible for the various forms of typhus. It is probable that persons with demonstrable tumors [2] represent but a fraction of those actually carrying the disease. Thus Theobald Smith's statement that "pathological manifestations are only incidents in a developing parasitism" may be true not only for the currently recognized contagious diseases but also for tumors.

In Cancer Families Breast Feeding Should Be Abandoned at Least for One Generation

Speaking with all reserve, there is reason to anticipate that the incidence of certain tumors at least, such as breast cancer in man, could be substantially reduced if the women of families with any tumors in their ancestry were to refrain entirely from nursing their progeny. A similar conclusion has been reached by Bittner (21).

Since no more than a few hours of breast feeding may suffice to transfer the tumor factor, the conclusion seems justified that breast feeding in such families should be abandoned *from birth,* and that artificial feeding should be substituted. It should be emphasized that many instances are known in experimental cancer research where females of tumor families of mice have been capable of transmitting the tumor factor without themselves displaying symptoms of the disease at any time.

Experiments previously reviewed suggest that it might suffice to omit breast nursing for one single generation. This simple preventive measure may bring substantial rewards in the fight against cancer, although results will not become evident until the next generation reaches the tumor age. Accurate records should be kept of persons, especially women, who have been artificially fed *from birth,* so that the incidence of tumors in them can be compared with either that of their ancestry or of the average population.

Probably all would agree that further research should be encouraged in the epidemiology of cancer and immunization against tumors.

[2] One hundred and fifty thousand people die each year of cancer in the United States alone (83).

Summary and Conclusions

Recent experiments demonstrate clearly that mammary cancer in mice is communicable from one generation to another. Animals transmitting the disease are, as a rule, carriers of a latent tumor factor and do not themselves display symptoms until they reach the "tumor age." The development of mammary cancer can be entirely avoided in susceptible mice by preventing newly born animals from nursing their potentially cancerous mothers.

The available data on accidental or intentional inoculation of human cancer are reviewed and the appearance of tumors in several members of the same or successive generations in man is discussed. The conclusion is suggested that human cancer may be similar to that observed in mice and may also, perhaps, be communicable from one generation to another.

Since milk seems mainly responsible for the transmission of certain tumors such as mammary carcinoma, it is suggested that the women of families with any malignant tumors in their ancestry refrain entirely from nursing their progeny. Artificial feeding should be substituted *from birth,* at least for one generation.

This simple preventive measure may bring substantial rewards in the fight against cancer, although results will not become evident until the next generation reaches the tumor age.

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Fluorescent Concentrates from the Nonsaponifiable Fractions of Human Livers*

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INTRODUCTION

The laboratory preparation of 20-methylcholanthrene from desoxycholic acid by Wieland and Dane (20) in 1933 encouraged speculation concerning the role that polynuclear aromatic hydrocarbons might play as endogenous carcinogenic agents, but in the succeeding decade progress in the characterization of endogenous chemical carcinogens has been extremely slow. Several investigators have attempted to demonstrate the existence of such agents, and the positive results described by des Ligneris (2); Hieger (4); Sannié and his co-workers (13, 14); Kleinenberg, Neufach, and Schabad (8, 9); and Steiner (17-19) suggest that chemical carcinogens may indeed be present in the fat-soluble extracts of certain human livers.

Should the presence of chemical carcinogenic agents in a specific tissue be established positively the isolation of the carcinogen would become of pressing interest, but progress in such work would be extremely slow if, at each stage of the fractionation, the potency of concentrates had to await assay by animal experimentation. One cannot, of course, assume that a carcinogen must be a polynuclear aromatic hydrocarbon merely on the grounds that it occurs in the nonsaponifiable fractions of liver extracts. The fact remains, however, that if such hydrocarbons are present in liver one would expect to find them in this fraction. It seemed to us worth while to try to develop fractionation technics suitable for the separation of polynuclear aromatic hydrocarbons from the commoner constituents of nonsaponifiable material.

Polynuclear aromatic hydrocarbons, both carcinogenic and noncarcinogenic, usually exhibit blue fluorescence and, at sufficiently high concentrations, give characteristic ultraviolet absorption spectra. Sannié, Truhaut, Guérin, and Guérin (13) examined the ultraviolet absorption spectra of the nonsaponifiable fractions of human liver, and reported no evidence of structure suggestive of the presence of benzpyrene or methylcholanthrene. Experiments carried out in our laboratories have shown that the nonsaponifiable fraction of fat-soluble extracts of human liver does contain material that exhibits blue fluorescence, and in this paper we discuss procedures we have developed for the concentration of the fluorescing component. The characterization of the fluorescing substance (or substances) is of interest, even should it prove to be unrelated to the postulated endogenous carcinogens. Statements have been made by Penn (12) that extracts can be obtained from cancerous human liver tissue that exhibit fluorescence similar to that of methylcholanthrene, but these assertions have been disputed by Hieger (5).

EXPERIMENTAL OBSERVATIONS

Methods and Materials

Fluorescent concentrates have been prepared from the nonsaponifiable fractions of 7 human livers; of these, 3 were from cancer patients showing no metastases to the liver, 3 from persons that were free of cancer, and 1 was from a patient with lymphosarcoma and hepatic necrosis.[1] Each liver was worked up separately, and fluorescent concentrates of an apparently similar nature were obtained from all of them. The initial saponifications were carried out by Steiner's method (17) and are described in the following section.

The nonsaponifiable fractions were subjected to various processes in attempts to concentrate the fluorescing component. Three of these are described below under Procedures I, II, and III. These all yielded pale yellow oils with strong greenish-blue fluorescence, readily soluble in petroleum ether but less soluble in ethanol. The ultraviolet absorption spectra of the final concentrates were similar, characterized by a maximum or plateau in the neighborhood of 2,550-2,600 A., with intensity ($E^{1\%}_{1\ cm.}$) of 30-100. The concentration of the fluorescing component was facilitated by the discovery of its specific adsorption on

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** At present on Active Service with the U. S. Army.

[1] We wish to thank Dr. A. Seligman, of The Beth Israel Hospital, Boston, Massachusetts, for supplying this clinical material.

picric acid from ethanol solution, and the method described in Procedure III, in which full advantage is taken of this fact, is considered the most satisfactory. The use of picric acid as a reagent for the fractionation of nonsaponifiable material from liver has been reported by Hieger (4) also.

Saponification of the Livers

The livers were minced immediately after autopsy, and stored under absolute ethanol at 5° C. pending further treatment.

In a typical case, to 790 gm. of minced liver in 1,000 ml. of absolute ethanol were added 1,000 ml. of a 10 per cent solution of aqueous potassium hydroxide. The mixture was heated on a steam bath in an all-glass apparatus for 25 hours, cooled to room temperature, diluted with water, and extracted 9 times with ethylene dichloride. The color of the initial extracts was deep orange, but the final extracts were almost colorless. The bulked extracts (2,000 ml.) were washed with distilled water, and the solvent was removed under reduced pressure in a current of nitrogen, leaving 11 gm. of red, oily residue. This residue was redissolved in ethanol (200 ml.) and refluxed with 200 ml. of 10 per cent aqueous potassium hydroxide solution for 6 hours. The cooled product was diluted with water, and extracted again with ethylene dichloride. The extract was washed with water, and on removal of the solvent under reduced pressure in a stream of nitrogen there remained 3.9 gm. of flaky, brick-red, crystalline solid. The several livers, or portions of liver, treated in this manner varied in weight from 790 to 2,400 gm. and yielded nonsaponifiable fractions of from 2.4 to 12.0 gm.

Fractionation of Nonsaponifiable Material

Procedure I.—Attempts were first made to remove hydroxylic material by esterification with succinic anhydride, under conditions favoring the formation of the alkali-soluble hemi-ester.

The red, nonsaponifiable material (3.9 gm.) was refluxed with 7.5 gm. of succinic anhydride and 5 ml. of pyridine in 50 ml. of dioxane for 3.5 hours. Water (25 ml.) was added and refluxing continued for a further 30 minutes. The cooled reaction product was diluted with 0.1 *N* sodium hydroxide solution and neutral constituents were extracted with ether. From the washed and dried ether layer 1.25 gm. of red oil was recovered. This was treated a second time with succinic anhydride as described above, and yielded 800 mgm. of neutral red oil.

Table I: Chromatographic Analysis of Red Oil Obtained in Procedure I

Fraction	Eluting agent: Volume	Eluting agent: Ethanol added to petroleum ether, per cent	Fluorescence of eluate	Residue on evaporation of eluate
1.	200 ml.	0.0%	nil	79 mgm. colorless oil
2.	200 "	0.0	"	21 " " "
3.	300 "	0.2	Blue (faint)	13 " " "
4.	150 "	0.2	" "	1 " " "
5.	200 "	0.4	" "	12 " " "
6.	100 "	0.6	nil	1 " " "
7.	200 "	0.6	"	5 " " "
8.	200 "	1.0	"	5 " " "
9.	200 "	2.0	"	4 " " "
10.	100 "	2.0	Blue (strong)	26 " yellow crystals
11.	200 "	3.0	" "	71 " " "
12.	250 "	3.0	" "	50 " " "
13.	100 "	3.0	" "	85 " oily orange crystals
14.	200 "	5.0	" (faint)	31 " yellow oil
15.	200 "	5.0	nil	19 " " "
16.	350 "	10.0	"	39 " " "
17.	200 "	100.0	"	25 " " "

Part of this red oil (680 mgm.) was dissolved in 200 ml. of petroleum ether and chromatographed on activated alumina on a column 30 cm. long and 1.4 cm. wide, under positive pressure (3), and fractionally eluted with petroleum ether-ethanol (1). A continuous yellowish-orange zone formed near the top of the column, with a sharply defined, colorless zone immediately beneath, which fluoresced a bright blue in ultraviolet light. With increasing concentration of ethanol in the eluting solvent, the orange (carotenoid) zone and the blue fluorescent zone traveled down the column without separating. Details of the chromatographic analysis are given in Table I, from which it will be noted that at least two fluorescent substances were present, one of which was not observed on the column and passed through with the early runnings. The fluorescence of this weakly adsorbed material was faint and these fractions have not been further investigated.

Fractions 10, 11, 12, and 13 contained the greater part of the more intensely fluorescing components together with carotenoid pigments, and sterols that had escaped esterification with succinic anhydride. These semicrystalline fractions were bulked, and weighed 232 mgm. On crystallization from ether 143 mgm. of material [2] separated out, the strong blue fluorescence remaining with the mother liquors.

The mother liquors were diluted to 25 ml. with ethanol and treated with 400 mgm. of digitonin in 25 ml. of ethanol. After the addition of 3 ml. of water some digitonide separated in a few hours, and was removed by centrifugation. The supernatant liquid retained its blue fluorescence and was diluted with water and extracted with ether. From the washed and dried ether extract 53 mgm. of a dark brownish oil were recovered, which showed green fluorescence. The ultraviolet absorption spectrum of this fraction is shown in Curve A of Fig. 1.

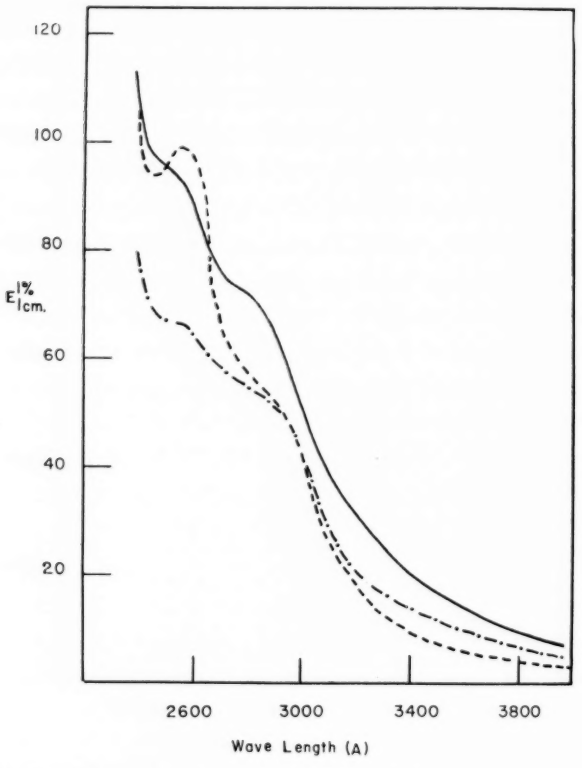

FIG. 1.—Ultraviolet absorption spectra of fluorescent extracts obtained by Procedure I. (Solvent ethanol.)

Curve A ——— Mother liquors from digitonin precipitation.
Curve B – – – – – Product recovered from picric acid adsorbate.
Curve C –·–·–· Product recovered from material not adsorbed on picric acid.

Part of this residue (47 mgm.) was treated with Girard's reagent to separate it into ketonic and non-ketonic fractions. The oil, dissolved in 0.3 ml. of glacial acetic acid, was treated with 250 mgm. of Girard's reagent. The solution was warmed on a steam bath for 5 minutes, cooled, and poured into 30 ml. of ice-cold water containing 0.207 gm. of sodium hydroxide. The non-ketonic material was extracted with 30 ml. of cold ethyl acetate. After removal of the ethyl acetate extract, the remaining aqueous phase was acidified by addition of 0.3 ml. of concentrated sulfuric acid and allowed to stand at room temperature for 5 minutes. The ketonic material was then extracted with ethyl acetate. The washed and dried ethyl acetate solution of the non-ketonic fraction yielded 40 mgm. of deep orange-colored oil with green fluorescence, while the ketonic fraction yielded 4 mgm. of nonfluorescing residue.

The fractionations described above should have eliminated from the fluorescing oil all β-3-hydroxy sterols, other hydroxylic material, and carbonyl compounds. Carotenoid pigments and their oxidative degradation products appeared to be the major components of the residual oil. The 40 mgm. of non-ketonic concentrate was dissolved in 2 ml. of ethanol and added to 500 mgm. of picric acid in 4 ml. of hot ethanol. The solution was allowed to cool to room temperature, and the picric acid that crystallized out was centrifuged down and removed. Another 500 mgm. of picric acid were added to the mother liquors and the mixture was warmed to dissolve the added solute. On cooling, a second crop of picric acid was obtained and added to that collected previously. The picric acid so obtained was dissolved in 10 per cent sodium carbonate solution and extracted repeatedly with ether. The ethereal solutions were combined, back extracted with sodium carbonate solution, washed with water, and dried with anhydrous sodium sulfate. On removal of the ether there remained 19 mgm. of yellow residue, obviously containing picric acid. This was dissolved in 2 ml. of ether, added to 50 ml. of petroleum ether, and adsorbed on a column of activated alumina. A dark yellowish-brown zone formed on the top of the column, but on development with petroleum ether containing 0.1 per cent ethanol, a colorless zone with strong blue fluorescence separated from this and moved about 1 cm. down the column. The column was partly dried by suction in a current of nitrogen, forced out of the glass tube, and the blue fluorescing zone sectioned out. After elution of the zone with ethanol and removal of the solvent there remained 6 mgm. of a pale yellow oil with intense greenish-blue fluorescence. The absorption spectrum is given in Curve B of Fig. 1.

The mother liquors from which the picric acid had

[2] This crystalline fraction melted over the range 128-131° C. After recrystallization from petroleum ether the melting point rose to 148.5-149° (corr.) and there was no melting point depression on admixture with cholesterol.

crystallized were processed in a manner similar to that described in the preceding paragraph for the crystalline picrate fraction. From the chromatographic column a fluorescent eluate was obtained, the spectrum of which is given in Curve C of Fig. 1. It is evident that separation of the fluorescent material by selective adsorption on picric acid, under the conditions described here, is not complete. Owing to insufficient material, no attempts have been made to fractionate this extract further. The procedure outlined above is summarized in Table II.

Procedure II.—In the first stage of Procedure I, the nonsaponifiable extract was refluxed in dioxane with pyridine and succinic anhydride. The conditions employed were rather drastic and the possibility could not be excluded that the fluorescent substance might be an artefact, produced during this reaction. In Procedure II this process was avoided, but the final concentrate obtained resembled very closely that obtained by Procedure I.

In Procedure II (See Table IV) fluorescent material was separated from sterols and other hydroxylic components by chromatographic analysis. The nonsaponifiable fraction (7.9 gm.) from the liver of a lymphosarcoma patient was chromatographed from petroleum ether, the column being developed with petroleum ether containing increasing amounts of ethanol. Details of the fractionation are given in Table III. As in the chromatographic separation described in Procedure I, two fluorescent components were observed; one, with weak blue fluorescence, in the early runnings from the column, and a more brilliantly blue fluorescing component accompanying the carotenoid fraction.

Fractions 13, 14, and 15, containing most of the more brilliantly fluorescent material, were bulked (729 mgm.) and rechromatographed on alumina from petroleum ether. After the column had been washed

Table II: Fractionation by Procedure I

LIVER (790 GM.)
Saponification
NONSAPONIFIABLE FRACTION (11.0 GM.)
Resaponification
NONSAPONIFIABLE FRACTION (3.9 GM.)
Esterification with succinic anhydride
NEUTRAL FRACTION (1.25 GM.)
Re-esterification with succinic anhydride
NEUTRAL FRACTION (800 MGM.)
Chromatographic analysis on activated alumina
FRACTIONS NO. 10-13 (231 MGM.)
Crystallization from ether
MOTHER LIQUORS (89 MGM.)
Precipitation with digitonin
MOTHER LIQUORS (53 MGM.)
Treatment with Girard's reagent
NON-KETONIC FRACTION (40 MGM.)
Adsorption on picric acid
ADSORBED FRACTION (6 MGM.)

Table IV: Fractionation by Procedure II

LIVER (1,800 GM.)
Saponification
NONSAPONIFIABLE FRACTION (11.0 GM.)
Resaponification
NONSAPONIFIABLE FRACTION (7.9 GM.)
Chromatographic analysis on activated alumina
FRACTIONS 13-15 (729 MGM.)
Chromatographic analysis on activated alumina
ELUATE FROM FLUORESCENT ZONE (167 MGM.)
Extraction with 83% ethanol
SOLUBLE FRACTION (35 MGM.)
Adsorption on picric acid
ADSORBED FRACTION (7 MGM.)

Table III: Chromatographic Analysis of Nonsaponifiable Fraction (Procedure II)

Fraction	Eluting agent: Volume	Eluting agent: Ethanol added to petroleum ether, per cent	Fluorescence of eluate	Residue on evaporation of eluate
1.	200 ml.	0.0%	blue (faint)	231 mgm. colorless oil
2.	250 "	0.0	" "	37 " " "
3.	150 "	0.0	" "	9 " " "
4.	200 "	0.0	" "	9 " " "
5.	125 "	0.0	green "	5 " " "
6.	125 "	0.1	" "	9 " " "
7.	200 "	0.1	nil	3 " " "
8.	100 "	0.1	"	2 " " "
9.	200 "	0.2	"	9 " pale yellow oil
10.	300 "	0.3	"	9 " colorless oil
11.	200 "	0.3	"	10 " " "
12.	200 "	0.3	"	4 " yellow "
13.	200 "	0.3	greenish-blue (strong)	93 " orange "
14.	100 "	0.3	" " "	305 " " "
15.	100 "	0.3	" " "	331 " " "
16.	100 "	0.3	" " (weak)	298 " " "
17.	175 "	0.3	" " "	905 " oily orange crystals
18.	500 "	0.3	nil	1,800 " pale yellow oil and white crystals
19.	400 "	0.3	"	2,000 " white crystals
20.	350 "	95.0	"	485 " deep orange oil

with 200 ml. of petroleum ether the fluorescing material formed a fairly sharp zone below the carotenoid layer; the chromatogram was partly dried in a current of nitrogen, pushed out from the glass tube, and the fluorescent zone sectioned out and eluted with petroleum ether containing 20 per cent ethanol. From this there were recovered 167 mgm. of a pale yellow oil, the spectrum of which is given in Curve A of Figure 2.

To 125 mgm. of this oil were added 100 ml. of 83 per cent aqueous ethanol and the mixture was agitated with a vigorous current of nitrogen for 15 minutes. The undissolved oil was allowed to settle, the supernatant ethanol removed by decantation and replaced by 100 ml. of fresh 83 per cent ethanol, and again agitated with nitrogen. This process was repeated 3 times and the combined ethanol extracts were evaporated to yield 35 mgm. of brown fluorescent oil. The residue that did not dissolve in 83 per cent ethanol weighed 92 mgm.; it also retained some of the blue fluorescence, but the ultraviolet absorption spectra indicated that most of the absorbing material had been selectively extracted.

The material soluble in 83 per cent ethanol was dissolved in 2 ml. of absolute ethanol and treated 3 times with 500 mgm. of picric acid as outlined under Procedure I. The neutral fraction remaining after extraction of the picric acid as sodium salt was chromatographed from petroleum ether on alumina. The fluorescent zone was sectioned from the partly dried column and yielded 7 mgm. of pale yellow oil with moderately intense blue fluorescence. The ultraviolet absorption spectrum of this concentrate is given in Curve B of Fig. 2 and resembles very closely the concentrate obtained by Procedure I, although the extinction coefficients are not so high.

Procedure III.—The procedure outlined in Table V has been developed more recently, and provides a simpler and less tedious method for the separation of the fluorescing material from sterols and carotenoid pigments. The bulk of the sterols is frozen out from petroleum ether solution, and most of the remainder removed as pyridonium sulfates by esterification with pyridine-sulfur trioxide at 55° C. (15, 16).

The nonsaponifiable material obtained from one-half of a liver was dissolved in petroleum ether and cooled with solid carbon dioxide. The sterol that crystallized out was filtered off and washed with cold petroleum ether, the washings being added to the mother liquors. The 4 gm. of crystalline material that remained was nonfluorescent and was discarded. The residue left on evaporation of the mother liquors was dissolved in 160 ml. of benzene and 8 gm. of pyridine-sulfur trioxide reagent (15) were added. The solution was heated at 55° C. for 1 hour, 1,300 ml. of petroleum ether were added, and the mixture was allowed to cool. The deposit that separated was filtered off and the filtrate, containing the non-hydroxylic material, was concentrated under reduced pressure in a stream of nitrogen to yield 3 gm. of residue.

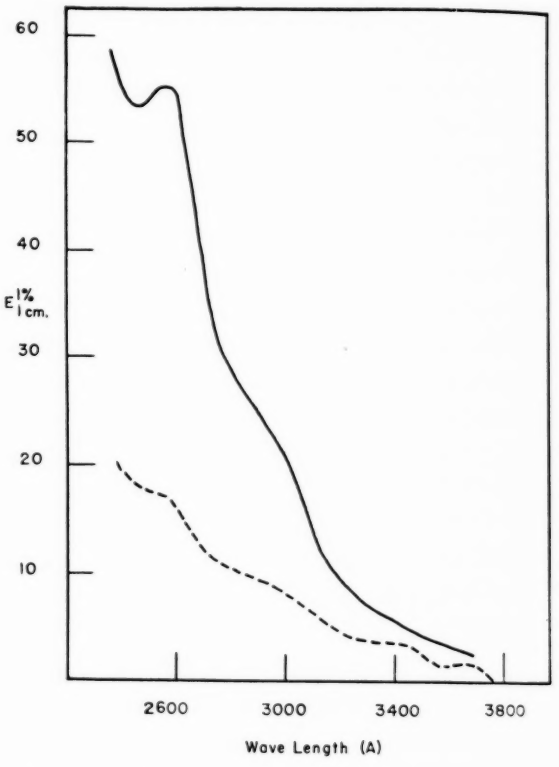

FIG. 2.—Ultraviolet absorption spectra of fluorescent extracts obtained by Procedure II. (Solvent ethanol.)

Curve A – – – – – Fluorescent fraction from second chromatographic analysis.

Curve B ——— Product recovered from picric acid adsorbate.

TABLE V: FRACTIONATION BY PROCEDURE III

LIVER (1200 GM.)
Saponified
NONSAPONIFIABLE FRACTION (9.9 GM.)
Resaponification
NONSAPONIFIABLE FRACTION (7.5 GM.)
Crystallized from cold ether
MOTHER LIQUORS (4.5 GM.)
Treated with pyridine-sulfur trioxide reagent
FRACTION SOLUBLE IN PETROLEUM ETHER (3.0 GM.)
Adsorption on picric acid
ADSORBED FRACTION (86 MGM.)
Added to 110 mgm. of similar material, washed with *N* sodium hydroxide solution and chromatographed on activated alumina.
FLUORESCENT ELUATE (33 MGM.)

This residue was dissolved in 20 ml. of ethanol and added to a hot saturated solution of 30 gm. of picric acid in ethanol. The solution was cooled to room temperature and filtered. A further 30 gm. of picric acid was added to the mother liquors, together with sufficient ethanol to bring the picric acid into solution at 70° to 80° C. The solution was again cooled and a

second crop of picric acid removed. This process was repeated a third time; the 3 batches of picric acid were combined, dissolved in ether-ethanol, and extracted with *N* sodium hydroxide solution until most of the picric acid was removed as sodium salt. The remaining ethereal layer was washed with water, dried with anhydrous sodium sulfate, and the solvent removed to leave a yellow oily residue.

This oil was dissolved in 200 ml. petroleum ether and chromatographed on activated alumina. A dark reddish brown zone formed at the top of the column, and a blue fluorescent zone immediately beneath. On development of the chromatogram with petroleum ether containing 0.5 per cent ethanol, the blue fluorescent zone moved down the column more rapidly than the reddish-brown zone and a fairly sharp separation was obtained. On removal of the solvent from the fluorescent eluate there remained 86 mgm. of pale yellow oil with strong greenish-blue fluorescence.

This material was added to 110 mgm. of similar oil obtained by the parallel treatment of the remainder of the same liver, and the combined fractions were dissolved in 50 ml. of petroleum ether and washed several times with *N* sodium hydroxide solution to remove traces of picric acid. The petroleum ether phase was then washed with water, dried with anhydrous sodium sulfate, and rechromatographed on activated alumina. A single blue fluorescent zone formed about 1 cm. below the top of the column. The chromatogram was washed with 500 ml. of petroleum ether and then with petroleum ether containing 1 per cent of ethanol. On addition of the alcoholic hexane, the fluorescent zone moved slowly down the column and yielded a colorless eluate with bright blue fluorescence, from which 33 mgm. of a very pale yellow oil was recovered. The spectrum of this oil is given in Fig. 3, and resembles very closely the spectrum of the oil obtained by Procedure II. Attempts to fractionate this oil further are at present under way.

Elemental Analyses of the Fluorescent Oils

Microanalyses for carbon, hydrogen, and nitrogen have been made on two samples of oil, one obtained by Procedure III and the other by a slight modification of Procedure II. The analytical data for the two samples agree fairly well (see Table VI). The hydrogen and the carbon together account for 95.1 per cent of the one sample and 96.4 per cent of the other, and no nitrogen could be detected in either sample.

Fluorescence Spectrum

The fluorescence of a sample of material prepared by Procedure III has been examined by Dr. J. A. Miller[3] who used the two-condition technic of fluorescence analysis developed recently by Miller and Baumann (11) for the determination of polynuclear aromatic hydrocarbons.[3]

Dr. Miller reports that in air, in solution in Skelly Solve B, the fluorescence intensity is 1/367th of that of an equal weight of 3,4-benzpyrene. The fluorescence spectrum, observed visually with a hand spectroscope, appears continuous from 5,850 to 4,440 A. ($\pm$50 A.).

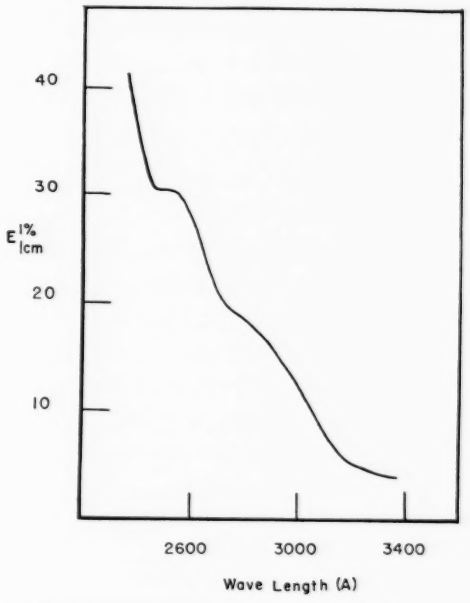

Fig. 3.—Ultraviolet absorption spectrum of fluorescent extract obtained by Procedure III. (Solvent ethanol.) Product recovered from picric acid adsorbate.

Table VI: Microanalyses of Fluorescent Concentrates

I. 3.332 mgm. of oil gave 3.661 mgm. of H_2O and 10.109 mgm. of CO_2
Found. C 82.80% H 12.29%
No nitrogen was evolved on analysis by micro-Dumas method.

II. 4.024 mgm. of oil gave 4.38 mgm. of H_2O and 12.42 mgm. of CO_2
Found. C 84.23% H 12.18%
8.658 mgm. of oil gave less than 0.01 ml. of nitrogen by micro-Dumas method.

The green part of the spectrum is most intense, but the weaker blue portion is definite. Determinations of the fluorescence intensity were made on solutions in 3 solvents, both in air and *in vacuo,* and the results are tabulated in Table VII. The appearance of the spectrum is similar to, but more intense than, that observed by Miller and Baumann for crude nonsaponifiable extracts of rat and mouse livers, while the vacuum/air intensity ratios agree closely with those

[3] We wish to thank Dr. Baumann and Dr. Miller for their kind cooperation in making these determinations.

observed for the rat and mouse liver fractions mentioned above. The vacuum/air ratios observed for most polynuclear aromatic hydrocarbons are much greater (11). The vacuum/air ratios for anthracene are comparable with those of the liver extracts, but the liver extracts lack the banded structure characteristic of anthracene in the blue region of the spectrum.

Recovery of Methylcholanthrene Added to Liver Tissue

It is suggested in the following discussion that if polynuclear aromatic hydrocarbons were present in liver they would be extracted from the nonsaponifiable material by adsorption on picric acid, in view of the readiness with which they form picrate complexes.

To test this experimentally, 5.2 mgm. of methylcholanthrene were added to 850 gm. of minced liver, which was then saponified by the regular method. The nonsaponifiable material (3.4 gm.) was chromatographed on alumina from petroleum ether, and after

Table VII: Fluorescent Intensity of Liver Extract Obtained by Procedure III

	Solvent		
	Skelly Solve B	Acetone	Pyridine
Vacuum	39.4 units	51.2	44.0
Air	20.1 "	30.6	34.1
Vacuum/air ratio	1.96:1	1.67:1	1.29:1

(Averaged for duplicate determinations: solutions contained 91 microgram per ml.: scale such that 0.1 microgram benzpyrene per ml. = 50 units.)

the column had been developed with 1,300 ml. of petroleum ether a bright, but diffuse, blue fluorescent zone was observed immediately beneath the main carotenoid zone. It was not practicable to separate the fluorescent zone from carotenoids and sterols by sectioning the column, and the sterol-carotenoid region together with fluorescing material was sectioned out and eluted with ethanol. The eluate yielded 2.6 gm. of semicrystalline material. By crystallization from 95 per cent ethanol, 1.4 gm. of nonfluorescent sterol was removed, and the mother liquors were then treated with 3 batches of 5 gm. of picric acid in the manner described previously. By solution of the picric acid adsorbate in *N* sodium hydroxide solution and extraction with ether a fluorescent fraction was obtained, which was freed from traces of picric acid by chromatographic adsorption; during the process a well-defined blue fluorescent zone formed on the column. From this, 63 mgm. of a pale yellow, oily residue was recovered, the spectrum of which is given in Curve A of Fig. 4. The two well-defined absorption maxima agree excellently in position with the two most prominent maxima in the spectrum of methylcholanthrene. The concentration of methylcholanthrene in the extract, calculated from the intensity of the maximum at 2,960 A., is 0.6 mgm., or about 11 per cent of the amount added to the liver. The actual recovery is somewhat less than this, as no correction is applied for the background absorption (6).

Purification of Reagents

Ethanol.—Absolute ethanol was used as supplied by the manufacturers without further treatment. Spectrographic tests were made of each fresh batch and there

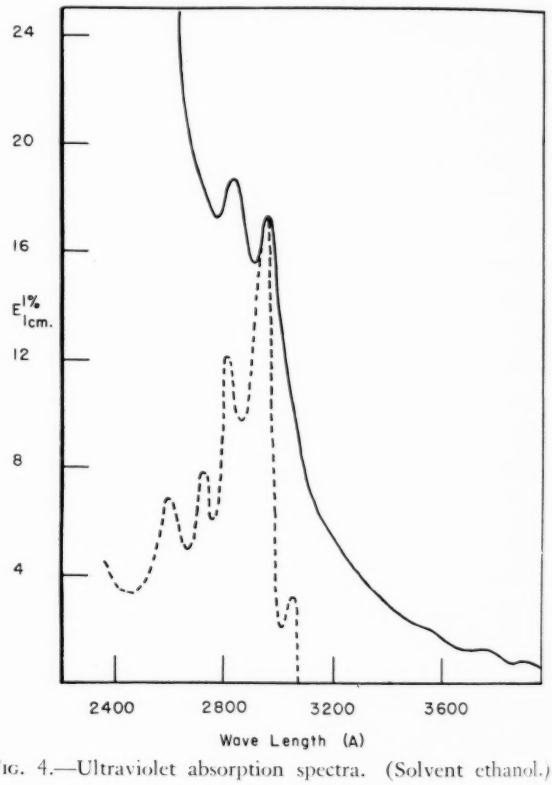

Fig. 4.—Ultraviolet absorption spectra. (Solvent ethanol.)

Curve A ——— Product recovered from picric acid adsorbate, liver with methylcholanthrene added.

Curve B – – – – – Curve calculated for pure methylcholanthrene.

was no characteristic absorption at wave lengths longer than 2,200 A.

Ethylene dichloride.—Technical grade material was washed twice with 5 per cent aqueous potassium hydroxide solution, twice with water, and distilled off anhydrous calcium chloride. This product was redistilled before use over a 36 inch fractionating column; b.p., 83.6° to 83.7° at 760 mm.

Petroleum ether.—Merck's petroleum ether (b.p., 30° to 60°) was shaken for 2 or 3 days with several changes of 10 per cent fuming sulfuric acid, washed with water, and then shaken again for several days with a saturated solution of potassium permanganate. The product was washed, and dried with anhydrous

calcium chloride and distilled over a 12 inch column packed with glass beads.

The ultraviolet absorption spectrum of the product showed no characteristic structure at wave lengths greater than 2,200 A. The residue remaining on evaporation of 200 ml. on a steam plate weighed less than 0.1 mgm. and, when dissolved in 5 ml. of ethanol this residue showed negligible absorption at wave lengths greater than 2,450 A. and faint continuous absorption between 2,450 and 2,200 A.

Picric Acid.—Eastman Kodak Reagent Grade was recrystallized once from ethanol. Ten grams of this material were dissolved in *N* sodium hydroxide solution and extracted repeatedly with ether. On evaporation of the ether a trace of nonfluorescing residue remained, but this yielded no fluorescent zone on chromatographic analysis from petroleum ether solution on activated alumina.

DISCUSSION

The pale yellow oils obtained by the three procedures described above show similar absorption spectra and fluorescence. Hydroxylic material was removed in Procedure I and III by chemical methods, and in Procedure I additional steps were taken to remove β-3-hydroxy sterols and carbonyl compounds. We have also observed that the fluorescing component of the oil is unaffected by refluxing with maleic anhydride in benzene solution for 3 hours, and there is no apparent change in the fluorescence of a petroleum ether solution of the oil when it is shaken with an aqueous solution of sodium hydrosulfite.

The adsorption on picric acid is suggestive of the behavior of polynuclear aromatic hydrocarbons, and the method employed for the separation of the adsorbed material from the adsorbate is essentially the same as that used for the fission of aromatic hydrocarbon-picric acid complexes. In the experimental section we describe a procedure in which 5.2 mgm. of methylcholanthrene were added to 850 mgm. of liver, which was then processed in a manner closely analogous to Procedure II. The fluorescent fraction recovered from the picric acid adsorbate possessed an absorption spectrum with two well-defined maxima at 2,830 and 2,960 A., characteristic of methylcholanthrene. The amount of methylcholanthrene recovered in this fraction was about 11 per cent of that added to the liver.

The ultraviolet absorption spectra of the fluorescent oils obtained by Procedures I, II, and III possess a maximum or plateau at 2,550 to 2,600 A. Whether or not the substance in the extracts that is responsible for this maximum is also the source of the blue fluorescence remains to be determined, but it is difficult to reconcile the observed spectrum with the presence of appreciable quantities of a polynuclear aromatic hydrocarbon. Solutions of many such hydrocarbons still fluoresce rather brilliantly at low concentrations, where the characteristic fine structure of the absorption spectrum is easily masked by background absorption, and the lack of structure in the spectrum cannot, therefore, be accepted as proof that such hydrocarbons are absent. At the same time, the lack of structure in the fluorescence spectrum, and the relatively weak quenching effect of oxygen on the intensity of the fluorescence, do not encourage the view that polynuclear aromatic hydrocarbons are responsible for the fluorescence. The analytical data (Table VI) suggest that the bulk of the material is probably of a hydrocarbon nature, and the analytical figures are not far removed from those required for squalene ($C_{30}H_{50}$ calc. C 87.7% H 12.3%).

Intense fluorescence is exhibited by many heterocyclic compounds, as well as aromatic hydrocarbons. Riboflavin, in particular, has an absorption maximum near 2,600 A., as have its photodecomposition products, lumiflavin and lumichrome, which also exhibit blue fluorescence (10). These alloxazine pigments are readily decomposed by alkali with liberation of urea, leaving degradation products that contain the weakly basic quinoxaline ring system, and it seems unlikely that such substances would survive the drastic saponification process. The properties of the known degradation products of riboflavin are hardly reconcilable with the observed chemical properties of these fluorescent oils. Failure to detect nitrogen on elemental quantitative analysis and to extract the fluorescent material from petroleum-ether solutions of the oils with *N* aqueous hydrochloric acid, would also seem to exclude basic heterocyclic compounds from consideration.

Further speculation about the nature of the fluorescing substances is not warranted on the basis of the available evidence and attempts at further fractionation are at present in progress.

SUMMARY

Three procedures are described for the preparation from the nonsaponifiable fraction of human livers of neutral oils exhibiting intense blue or bluish-green fluorescence. Concentrates of a grossly similar character have been obtained from healthy livers, livers of cancer patients showing no liver metastases, and a case of lymphosarcoma and hepatic necrosis. The concentrates obtained by the three different methods all have ultraviolet absorption spectra with a maximum or plateau at 2,550 to 2,600 A., but neither the ultraviolet nor the fluorescence spectra show fine-banded structure of the type associated with polynuclear aro-

matic hydrocarbons. The quenching effect of oxygen on the intensity of fluorescence is less than that usually observed for solutions of such hydrocarbons.

The fluorescing component of the concentrate exhibits considerable chemical stability. It is not affected by *N* aqueous hydrochloric acid or by alkali, nor does it react with reagents that attack the hydroxyl group (succinic anhydride, pyridine-sulfur trioxide), or with digitonin, Girard's reagent, maleic anhydride, or an aqueous solution of sodium hydrosulfite. It appears to be adsorbed specifically on picric acid from an ethanolic solution. Although the spectrographic data do not encourage the view that the fluorescing substance is a polynuclear aromatic hydrocarbon, it has been demonstrated that methylcholanthrene, when added to a liver, accumulates in the same fraction.

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Oral Cancer in Bombay, India. A Review of 1,000 Consecutive Cases*

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(From the Tata Memorial Hospital for the Treatment of Cancer and Allied Diseases, Bombay, India)

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A study of the incidence of cancer according to sites among the various human races may be useful for an elucidation of some of the factors responsible for their causation. This would be so particularly if those under investigation possess notable differences in their habits, customs, occupational pursuits, and stages of economic development. The different communities in India present exceptional opportunities for an investigation of this character as there is still hardly any intermarriage between the different castes and communities. Even when economic necessity, industrialization, and urban concentration cause people to migrate from one part of the country to another, every effort is made by them to retain their original dietary habits, culinary details, and original groupings. Thus a division of the people according to religious sects, groups, and subgroups, that bewilders the foreigner, affords interesting human material for observation, analysis, and reflection under almost experimental conditions.

The following data relating to a single group of diseases observed in patients who registered themselves for treatment during the 30 months from March, 1941, to September, 1943, at the Tata Memorial Hospital, Bombay, are therefore presented below with a view to making such a study. During this period 4,765 were found to be suffering from neoplastic disease. The cancer was located in the buccal cavity in 1,000 out of a total of 2,880 cases of carcinoma. Only those cases that showed a positive biopsy are included in this review.

It is seen (Table I) that in over 60 per cent of the cases in Bombay the disease was situated at the base of the tongue and in the tonsils. The next largest group comprises cancer of the cheek, the floor of the mouth, and the alveolus, accounting for 22.5 per cent of the total. Cancer of the lip and the anterior two-thirds of the tongue was relatively infrequent. A comparison between the figures at the two hospitals in Southern India (Bombay and Neyoor) and the Memorial Hospital, New York, as regards the proportional distribution of malignant neoplastic disease in different regions of the mouth presents interesting features, as is shown by the same table and by Figs. 1 and 2.

(a) It will be seen that cancer of the lip accounts for nearly 20 per cent of the oral cancer at the Memorial Hospital, in New York, and for only 1.7 per cent at the Tata Memorial Hospital, in Bombay.

TABLE I: DISTRIBUTION OF INTRAORAL CANCER ACCORDING TO SITES

	Bombay, India, Tata Mem. Hospital Mar., 1941-Sept., 1943			New York, U. S. A. (1), Memorial Hospital 1917-1929			Neyoor, S. India (2), London Mission Hospital	Manila, P. I. (3),
	Male	Female	Total	Male	Female	Total		
Lip	10	7	17	555	34	589	40	3
Buccal mucosa	111	54	165	324	38	362	296	49
Floor of mouth	19	4	23	260	14	274	196	1 *
Alveolus	24	13	37	151	22	173	41	3 †
Tongue	479	43	522	685	103	788	96	13
Anterior $\frac{2}{3}$	79	16	95	...	...	525 ‡	..	..
Base	400	27	427	..	..	263 ‡	..	..
Tonsil	159	15	174	335	34	369	..	3
Palate	56	6	62	203	104	307	..	3
Total	858	142	1,000	2,513	349	2,862	669	75

* Lower jaw.

† Upper jaw.

‡ These are approximate figures based on a study of 673 cases of carcinoma of the tongue at the Memorial Hospital by Hayes Martin (10).

* Because of the difficulties of international communication the author has not read proof of this article.

The cancer mortality figures for the United States of America (4) show that the high incidence is not due to an unusual attendance at the Memorial Hospital of those suffering from this type of cancer (Table II).

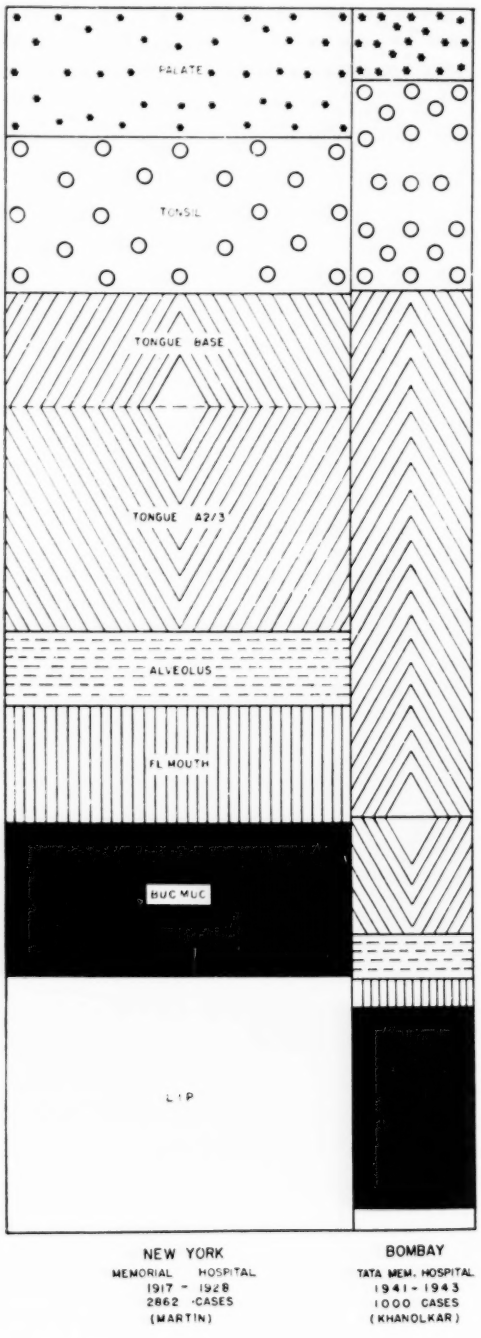

FIG. 1

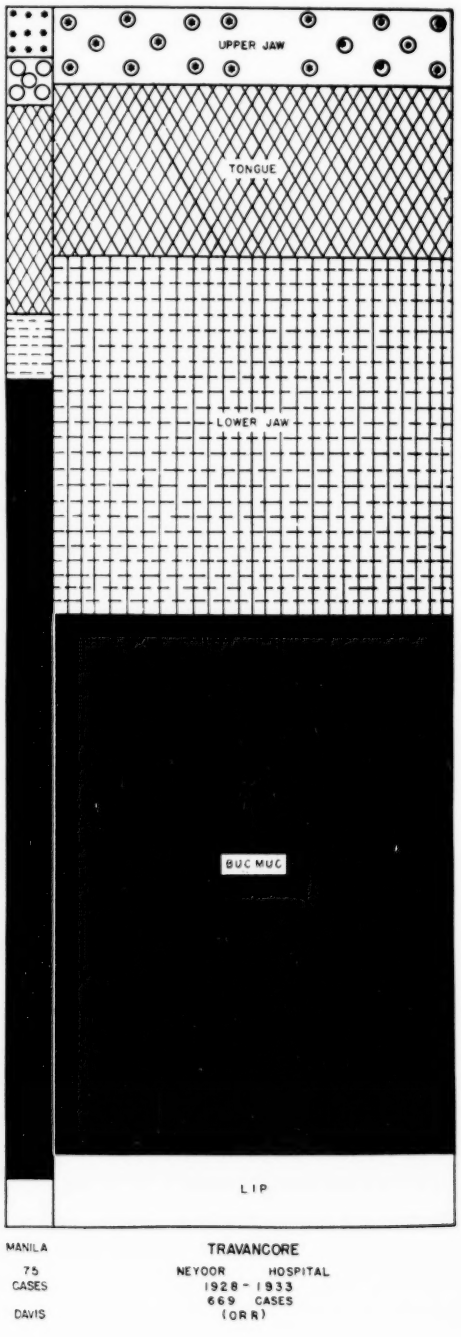

FIG. 2

(b) In New York cancer of the anterior two-thirds of the tongue is twice as common as cancer at the base (10), whereas the proportions reveal a reverse tendency in Bombay.

The inhabitants of the latter city can be broadly divided into Indians and non-Indians. The Indians are mainly Hindus, Muslims, Christians, and Parsees. The bulk of Hindus (51 per cent of the population) are Deccanis (5), and they belong to Bombay and the districts east and south of the city. The Gujaratis form the next largest group (20 per cent of the population), and have migrated from the districts to the north of the city along the western seaboard.

Table III and Fig. 3 classify the figures according to the communities in Bombay and reveal the following peculiarities:

1. As compared to that in the Gujaratis, cancer of the buccal mucosa is 6 times (5.2 per cent against

30.8 per cent) more common among Deccanis, while cancer of the tongue is 1½ times as common among the Gujaratis (64.3 per cent against 42.8 per cent), the majority of this higher incidence affecting the posterior third of the tongue.

2. The Muslims who migrate to Bombay from the Deccan and the Gujarat occupy an intermediate position as regards both these types of cancer.

DISCUSSION

Most of the recent publications on medical statistics have emphasized the inherent shortcomings of conclusions based on hospital data regarding the morbidity or mortality due to any particular disease. It has been argued that such data cannot be wholly accepted even as an index to a geographical distribution of that disease. Willis (20), while reviewing 500 consecutive autopsies on tumor patients, has referred to the fallacies arising as a result of drawing uncritical statistical deductions from the records of a single institution or a group of institutions. He has pointed out that such records "usually fail to afford average samples of the whole community; in many ways, seen and unseen, their material is of a selected kind." At the same time it would be equally wrong to reject such data completely, for they help to indicate the relative importance of certain diseases in certain populations in spite of the limitations imposed upon them by differences in sex and age groups, and the eagerness and opportunity of the people to seek relief in medical institutions. These limitations have been referred to by Bonne (2) in his study of cancer in the inhabitants of Java. It may therefore be necessary to state some of the factors that may unbalance a study of this nature, and to mention a few facts regarding the population from which patients at this hospital are drawn.

Bombay is a relatively modern cosmopolitan port through which a large proportion of imports and exports of the subcontinent of India filter in and out. At the last census (1941) its population was reported to be 1,489,883 (5). Because of the paucity of modern centers of medical relief in the country it draws many

TABLE II: CANCER MORTALITY IN THE UNITED STATES OF AMERICA FOR 1935 AND 1936

	1935		1936	
Cancer of the Buccal Cavity	M 3,232	F 720	M 3,303	F 778
Lip	671 (20.76)	56 (7.77)	681 (20.61)	83 (10.66)
Tongue	878 (27.16)	198 (27.50)	887 (26.85)	210 (26.99)
Mouth	441 (13.64)	109 (15.13)	487 (14.74)	133 (17.09)
Jaw	776 (24.00)	223 (30.97)	724 (21.91)	226 (29.04)
Other and unspecified parts of buccal cavity.	466 (14.41)	134 (18.61)	524 (15.86)	126 (16.19)

TABLE III: 1,000 CASES OF ORAL CANCER IN 4,769 PATIENTS

1941-1943

Hosp. attendance %	Community		Lip	Buccal mucosa	Floor of mouth	Alveolus	Tongue, anterior 2/3	Tongue, base	Tonsil	Palate	Total	Carcinoma # 1-4769	A, %	Malignant neoplastic disease # 1-4769	B, %
29.81	Gujarathis	M	1	13	2	4	17	200	72	21	330	555	59.45	580	56.90
		F	1	5	..	2	3	2	2	..	15	108	13.80	118	12.71
27.47	Deccanis	M	3	50	6	7	18	68	25	11	188	508	37.00	560	33.57
		F	3	24	1	4	4	13	3	1	53	352	15.05	373	14.20
6.1	Other Hindus	M	1	16	..	2	9	19	17	2	66	237	27.84	266	24.81
		F	..	5	..	2	3	1	..	1	12	94	12.76	101	11.88
26.2	Muslims	M	2	26	5	9	24	89	33	16	204	429	47.55	455	44.84
		F	2	18	..	2	2	5	8	2	39	133	29.32	139	28.06
5.8	Christians	M	1	3	5	..	2	14	11	2	38	93	40.86	104	36.54
		F	1	1	1	2	2	4	2	1	14	110	12.72	120	11.66
2.4	Parsees	M	..	3	1	1	5	3	..	2	15	45	33.33	57	26.32
		F	..	..	2	1	2	1	..	..	6	98	6.12	104	5.77
1.8	Non-Indians	M	2	..	..	1	4	7	1	2	17	67	25.37	86	19.77
		F	..	1	..	..	..	1	..	1	3	51	5.88	55	5.45
		M	10	111	19	24	79	400	159	56	858	1,934	44.36	2,108	40.70
		F	7	54	4	13	16	27	15	6	142	946	15.01	1,010	14.06
	Total		17	165	23	37	95	427	174	62	1,000	2,880	34.72	3,118	32.07

A—Represents the percentage of oral carcinoma to total carcinoma.

B—Represents the percentage of oral carcinoma to total malignant neoplastic disease.

Carcinoma	—2,880
Total malignant neoplastic disease	—3,118
Benign tumors	— 365
Undiagnosed	— 26
Non-neoplastic	—1,260

patients from long distances. The population by communities is tabulated as follows:

Hindus	1,061,047	Parsees	59,813
Muslims	251,318	Europeans	21,798
Christians	78,149		

reports showing the proportion of the population by languages (5).

Most of the patients are referred to Tata Memorial Hospital with a provisional diagnosis of cancer by medical practitioners. Under such conditions those

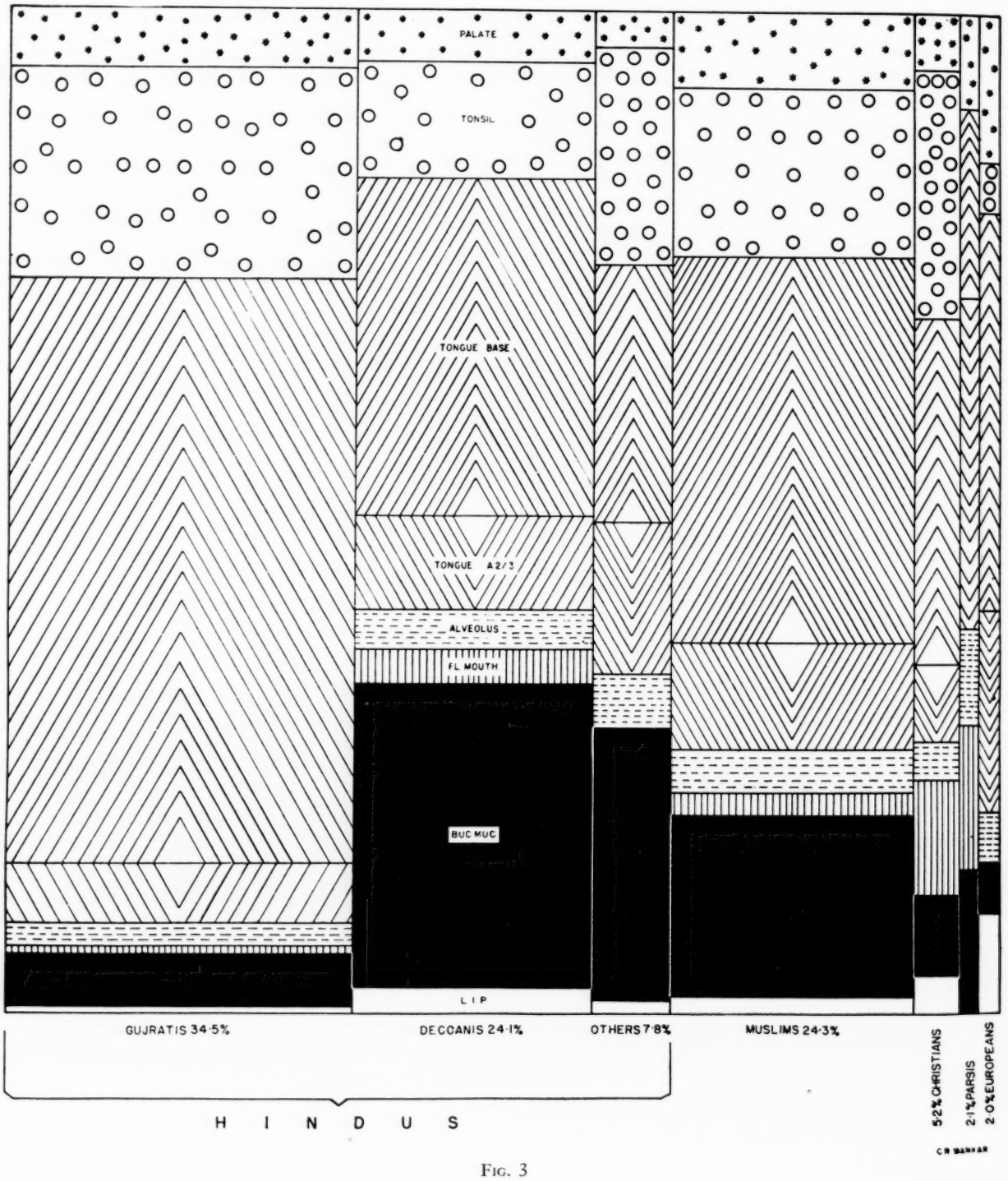

FIG. 3

The two main regional groups of Hindus are Deccanis and Gujaratis, and are in the proportion of 2.5:1 as judged from the attendance of children at the city's schools (1), and 2:1 from a statement in the census

neoplastic diseases that are easily diagnosed, like cancers on the visible surface of the body (skin, mouth, etc.), will be relatively more numerous than tumors in the internal organs, whose bearers find their way to

large general hospitals and are treated there. This discrepancy obtains in all countries where there is a competition between general and special hospitals, and the data presented here are therefore comparable with reports from similar medical institutions elsewhere.

The population of the city can be roughly classified into five main groups. This grouping is somewhat unsatisfactory as the Hindus consist of many subgroups that are both ethnically and geographically different. A majority of Muslims and Christians have until recently lived like the subgroups of Hindus from whom they were derived. A regional subdivision of the Hindus into Deccanis, Gujaratis, and others is easily understood by the people in the city. It is, however, open to obvious criticism inasmuch as the caste groups found within each region are endogamous and each group comprises populations at different stages of economic well-being and culture. This results in differences in dietary and other habits, even though there may be a general and apparent similarity in the pattern. The justification for adopting such a grouping lies in the fact that the members of the groups do not intermarry. Although the individual castes within the groups form endogamous divisions, yet according to informed opinion the castes within each region are racially more akin to each other than similar castes in other regions. The socioeconomic background of the two regions is distinct. There is considerable similarity between the different castes of the same regions in matters of dress, food, etc.

The incidence of mouth cancer, as will be seen from Table III, varies greatly in different parts of India. This table is compiled from data collected by Vishwanath and Grewal (19) from teaching hospitals in the principal centers in India. It will be seen that this type of cancer is lowest in the Punjab (4.5 per cent) and highest in Madras (34.5 per cent). At Neyoor, in Travancore, Somervell (16) records that the staff of the London Mission Hospital performed 7,445 operations for oral cancer out of a total of 10,125 operations for carcinoma at all sites (73 per cent) in the last 20 years. The corresponding figure in European countries is probably in the vicinity of 7 per cent. The number of cases of mouth cancer in the hospitals in the County of London in the years 1938 and 1939 were 529 and 489 respectively, while the total cases of cancer registered in those hospitals in the same years were 7,867 and 7,201 respectively (3).

At the Memorial Hospital, New York, there were 2,862 cases of oral cancer out of a total of 16,565, or 17.27 per cent, during the inclusive period 1917 to 1929 (12). This is a higher rate than that encountered in London hospitals.

Hoffman (8) has given the percentage of deaths from cancer of the buccal cavity to all forms of cancer in the following countries as shown in Table V.

The contrast in the distribution of lip and tongue cancer between Bombay and New York, which differ considerably in their climatic and other conditions of life, raises problems that deserve further consideration. It is likely that "certain external factors operating through man's ordinary environment may be of great importance in determining in what part of the body cancer will appear" (17). The reason for the difference in the incidence of oral cancer in different regions of India has been attributed to the habit of betel leaf chewing. The leaves, the nut, and the other ingredients used in the Philippines are described in detail by Davis (6), and do not differ much from those in use in India. The habit is widespread throughout the country though its extent varies considerably in different parts. In the Punjab, for instance, it is least prevalent, and becomes more noticeable as one proceeds east and south. The precise effect of this habit on the production of mouth cancer has raised a great deal of controversy among those who have had an opportunity of studying this condition. As the figures in Table IV show, however, the habitual chewing of betel leaf and areca nut, except in so far as it leads to deficient oral hygiene, has no other etiological role in the production of mouth cancer. In Bengal the habit is very common, and yet the incidence of oral cancer is next to the lowest in India (Fig. 4).

In most parts of India many adults chew the betel leaf and the areca nut with some type of dried tobacco powder. When one considers the use of chewing tobacco along with betel leaf, the position regarding the development of mouth cancer becomes somewhat different. It is the opinion of those who have had wide experience amongst people who chew betel with tobacco that such a habit is closely associated with the development of cancer of the mouth. Somervell (16), on the basis of long experience and a clinical survey carried out by his colleague, Orr (11), has arrived at the following conclusions:

"I am satisfied that the following are the main etiological factors causing epitheliomas of the mouth in South India. Chemical irritations due to alkaloids produced by the action of lime on the tobacco chewed with the betel leaf and areca nut. The more prolonged this irritation, the more likely is it to lead to epithelioma. The use of lime made from shells is more injurious than is the lime made from limestone owing to its fine division and rapid setting-free of alkaloids from tobacco. Certain kinds of tobacco notably the strong vadakkan tobacco of South India and the tobacco of Jaffna, Ceylon, are more likely to cause cancer of the mouth than are the milder tobaccos." He

TABLE IV: INCIDENCE OF MALIGNANT NEOPLASTIC DISEASE FROM HOSPITAL REPORTS

	Total inpatients	Malignant disease	Oral cancer	Hindu		Muslim	
				Male	Female	Male	Female
Bombay (Bombay city)	153,260	2,896 (1.89)	607 (20.95)	297/1,192 (24.91)	62/711 (8.72)	139/404 (34.40)	28/138 (20.28)
Madras (Madras city) (Ahmedabad, Poona, & Miraj)	163,470	4,922 (3.01)	1,697 (34.47)	997/2,002 (49.80)	338/1,954 (17.29)	138/225 (61.33)	27/87 (30.22)
Bengal (Calcutta)	219,540	2,401 (1.09)	193 (8.04)	96/975 (9.84)	38/743 (5.11)	34/321 (10.59)	12/112 (10.71)
Punjab (Lahore & Ludhiana)	64,118	1,822 (2.84)	82 (4.50)	21/363 (5.78)	11/749 (1.46)	30/258 (11.62)	15/279 (5.36)
United Provinces (Lucknow & Agra)	79,936	2,375 (2.97)	325 (13.68)	157/1,033 (15.19)	37/708 (5.22)	72/305 (23.60)	49/234 (20.94)
Bihar & Orissa (Patna, Cuttack, & Darbhanga)	84,964	1,735 (2.03)	361 (20.92)	252/1,107 (22.76)	45/434 (10.36)	33/121 (27.27)	18/101 (17.82)

Figures in parentheses represent percentages. The figures under Hindus and Muslims denote oral cancer/total malignant neoplastic disease.

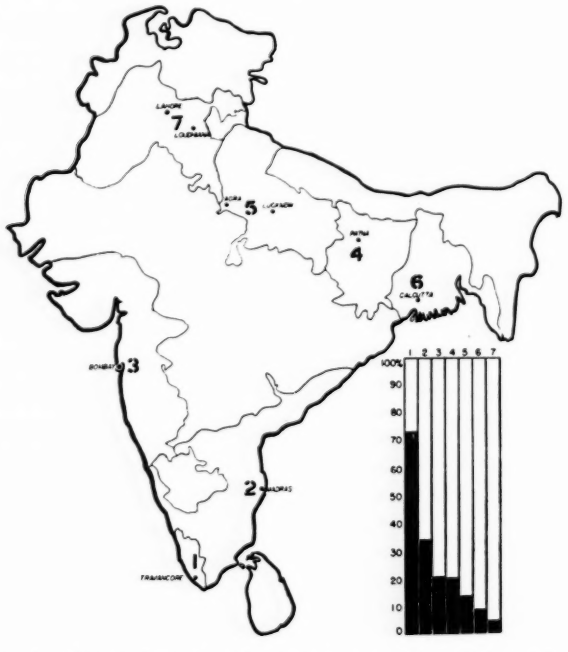

FIG. 4.—Comparison of oral cancer in seven large cities of India.

The solid black areas show the proportion of oral cancer in the chief hospitals in the seven cities as percentage of total cancer at those institutions. The numbers at the top of the histogram correspond to the numbers on the map that denote the capital cities of the different provinces.

1. Travancore
2. Madras
3. Bombay
4. Patna
5. Agra-Lucknow
6. Calcutta
7. Lahore—Loudhiana

further states "Of more real value than any set of figures are the general impressions one has obtained in the course of 20 years," and he is proud of the fact that experience rather than statistics has been the foundation of his remarks. Unfortunately these categorical assertions are unsupported by any attempt at isolation of the supposed carcinogenic alkaloids in the betel chew, with or without tobacco, or by any experimental

TABLE V: PERCENTAGE OF DEATHS FROM CANCER OF THE BUCCAL CAVITY TO ALL FORMS OF CANCER FOR DIFFERENT COUNTRIES

	Male	Female
Egypt	2.5	1.0
Japan	3.4	1.3
Hawaii	3.7	0.6
Singapore	5.1	3.8
U. S. A., Negroes	5.6	1.3
U. S. A., Indians	5.5	1.5
Barbados	8.5	1.3
Cuba, Colored	12.7	6.3
Cuba, White	14.8	5.1
Philippine Islands	22.9	13.6
Ceylon	50.4	25.3

investigations tending to a production of cancer in animals. Like many clinical impressions the suggestions put forward by Orr and Somervell may constitute a discovery of some significance. It would be difficult, however, to accept their impressions without further study in spite of the fact that medical history abounds in flashes of clinical insight. Two further points of interest emerge from the observations of Orr:

1. The laborers on the tea estates in the hills of central and southern Travancore have migrated from the plains and belong to the same race as the plainsmen on the coast. They chew the same betel, lime, and tobacco mixture, yet oral cancer is reported to be scarcely known amongst them. This freedom of the estate laborer is attributed by Orr to an improved economic status and the more varied diet that they can just afford on an economic security of 15 to 20 cents a day by way of wages.

2. Oral cancer is reported to be extremely rare among the Hindus of Bihar, although they are seen to be chewing betel incessantly. It is, however, fairly common among the Santals, an aboriginal tribe who do not use betel or areca nut but chew a tobacco and lime mixture. The Santals eke out a precarious living in the wooded hills of Bihar and their diet is probably

very deficient in many essential constituents for the same reasons.

It has been pointed out by Friedell and Rosenthal (7) that a perusal of the controversy regarding the etiological role of betel leaf chewing in the production of mouth cancer reveals the fact that reports from the countries that fail to show a greater incidence of cancer of the mouth in betel chewers, also fail to mention the use of tobacco in describing the preparation of the betel chew. It will be seen from Davis' description (6) that Philippinos use either the American plug tobacco or a cheaper local variety along with the betel leaf. The prevalence of oral cancer in Philippinos is shown in Table I and Fig. 2.

The remarkable frequency with which cancer of the base of the tongue occurs in the inhabitants of Western India, and particularly in Gujarati males, needs further study and elucidation. The traumatic and irritative factors such as carious and jagged teeth, which have an etiological role assigned to them in the production of cancer of the anterior two-thirds of the tongue, have no part in the causation of this condition in Bombay. It is at present too premature to enter into any speculative hypothesis. "A list of the human tumors that have been traced to the action of provocative carcinogens is in no small degree a sociological document, reflecting as it does the ways of life, vocations, avocations, habits, and environmental stresses of peoples and individuals" (14).

The series of experimental investigations on the carcinogenic action of tobacco and its derivatives have not yielded so far any clear-cut results. The assertions of Roffo (13) regarding the production of squamous carcinoma on the skins of rabbits have not been confirmed by Sugiura (18). There is a general impression, based on clinical observation, that a long-continued use of tobacco is conducive to the production of mouth cancer in susceptible persons, but it will be evident that this impression needs to be supported by careful and controlled experimental work. The observations of Lu (9) and of Schürch and Winterstein (15) regarding the contributory role of cholesterol in the production of squamous epithelioma in rabbits after painting with tobacco derivatives deserves further exploration.

This review will have served its purpose if it succeeds in showing that there is a problem, and that a proper understanding of the factors leading to the development of a common type of carcinoma in Western India may result in the avoidance of much suffering.

SUMMARY

One thousand consecutive cases of oral cancer seen at the Tata Memorial Hospital during the past 30 months have been reviewed.

Differences in the distribution of oral cancer according to site between communities in Bombay and between similar data at other hospitals have been pointed out and discussed.

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American Association for Cancer Research, Inc.

Special Meeting of the Executive Committee

January 12, 1944

A special meeting of the Executive Committee was held at 333 Cedar Street, New Haven, Connecticut, on January 12, 1944. The meeting was called by order of Dr. James B. Murphy, Chairman of the Executive Committee. Drs. J. B. Murphy, G. M. Smith and W. U. Gardner were present. The Executive Committee had been authorized to function in the absence of a meeting of the Board of Directors as a result of a poll of the members of the Board of Directors submitted on October 23, 1943. A majority of the directors voted that the affairs of the Association be entrusted to the Executive Committee appointed by the President at the last meeting of the Board of Directors.

Because this was the first meeting of the Executive Committee there were no minutes, old business, or reports of special or standing committees.

NEW BUSINESS

Annual Meeting

The Executive Committee concurred uniformly with the results of the poll of the Board of Directors that the Thirty-Sixth Annual Meeting of the members of the Association be postponed during the year of 1944.

The Secretary presented the names of seven former members whose deaths were reported during the past year. The Executive Committee expressed its profound sorrow at this great loss and voted that the names of the deceased be recorded in the minutes of the meeting.

Carl W. Apfelbach, M.D., Rush Medical College, 1758 West Harrison Street, Chicago, Illinois. Died June 25, 1943.

James Ewing, Sc.D., M.D., The Memorial Hospital, York Avenue at 68th Street, New York, N. Y. Died May 17, 1943.

Francis P. Garvan, 654 Madison Avenue, New York, N. Y. (Honorary Member.)

Mataro Nagayo, M.D., Japanese Foundation for Cancer Research, Nishi Sugamo Tosimaku, Tokyo, Japan.

Frederick J. Taussig, M.D., 3720 Washington Boulevard, St. Louis, Missouri. Died August 21, 1943.

H. Gideon Wells, M.D., Ph.D., University of Chicago School of Medicine, Chicago, Illinois. Died April 26, 1943.

Louis B. Wilson, M.D., Mayo Clinic, Rochester, Minnesota. Died October 5, 1943.

Action Pertaining to Members

Resignations of seven members of the Association were presented and accepted.

Berrill, Norman J., Ph.D., McGill University, Montreal, Canada.

Brown, Samuel, M.D., University of Cincinnati, 707 Race Street, Cincinnati, Ohio.

Burr, Harold S., Ph.D., Yale University School of Medicine, 333 Cedar Street, New Haven, Connecticut.

Eberly, Albert O., M.D., Mt. Morris Tuberculosis Hospital, Mt. Morris, New York.

Hoffman, Frederick L., LL.D., Biochemical Research Foundation of the Franklin Institute, San Diego, California.

Jackson, Henry, Jr., M.D., Collis P. Huntington Memorial Hospital, 695 Huntington Avenue, Boston, Massachusetts.

Page, Irving H., M.D., Eli Lilly & Company, Indianapolis, Indiana.

The membership of nine members of the Association was discontinued in accordance with action taken by the Board of Directors.

Bachman, Werner E., Ph.D., University of Michigan, Department of Chemistry, Ann Arbor, Michigan.

Davis, William W., Ph.D., Eli Lilly & Company, Indianapolis, Indiana.

Gaspar, Istvan, M.D., Rochester General Hospital, Rochester, New York.

Jetter, Walter, M.D., Harvard University, Boston, Massachusetts.

Miller, David K., M.D., E. J. Meyer Memorial Hospital, 462 Grider Street, Buffalo, New York.

Murphy, Walter T., M.D., 113 High Street, Buffalo, New York.

Murray, Joseph M., Ph.D., University of Maine, Orono, Maine.

Nolan, Lewis E., M.D., Laird Memorial Clinic, Montgomery, West Virginia.

O'Brien, Joseph P., M.D., 749 South Park Avenue, Buffalo, New York.

Nominations of eleven applicants for membership in the Association were presented and the applicants were duly elected to membership.

Bass, Allan D., M.D., Vanderbilt University, Nashville, Tennessee.

Burmester, B. R., Ph.D., Regional Poultry Research Laboratory, East Lansing, Michigan.

Deringer, Margaret K., Ph.D., National Cancer Institute, Bethesda, Maryland.

Gottschalk, R. G., M.D., Children's Hospital, 300 Longwood Avenue, Boston, Massachusetts.

Herly, Louis, M.D., 440 West End Avenue, New York, N. Y.

Heilman, Fordyce R., M.D., Mayo Foundation, University of Minnesota, Minneapolis, Minnesota.

Millan, Ignacio, M.D., Director Cancer Clinic General Hospital, Avenue Veracruz 69, Mexico City, Mexico.

Prickett, Cavett O., D.V.M., Regional Poultry Research Laboratory, East Lansing, Michigan.

Tavares, C. A., M.D., Station Hospital, Camp Anza, Arlington, California.

Williams, Roger J., Ph.D., D.Sc., University of Texas, Austin, Texas.

Wilson, Hugh M., M.D., Yale University School of Medicine, New Haven, Connecticut.

Two members of the Association were elected honorary members.

CHILDS, STARLING W., 1 Wall Street, New York, N. Y.
HARRISON, Ross G., PH.D., M.D., Yale University, New Haven, Connecticut.

REPORT OF THE TREASURER

The report of the Treasurer was read and approved. The Treasurer was instructed to submit this report to Dr. Burton G. Simpson, auditor for the Association.

CANCER RESEARCH

A motion was duly made and voted that the Secretary resubmit the resolution drawn up during the past year by Dr. C. C. Little, Chairman of the Journal Committee, to the several research foundations contributing to the journal, *Cancer Research,* expressing appreciation for this generous assistance and stating that it is the desire of the Association to assume eventually a greater responsibility in the financial support of the journal. This resolution was published in *Cancer Research,* 1943, Volume 3, page 476.

It was duly voted that the Secretary-Treasurer be authorized to pay to the journal *Cancer Research* the sum of $1.00 per member and an additional amount so that a total of $500.00 will be contributed by the Association for the current year (January 1, 1944 to December 31, 1944). This action continues the policy adopted last year at the meeting of the Board of Directors held in New York City on February 12, 1943.

PUBLICATIONS

It was duly voted that the Secretary-Treasurer be authorized to submit the proceedings of this meeting for publication in *Cancer Research* and to pay expenses incurred by such publication.

The Secretary-Treasurer was authorized by motion duly made, seconded, and voted to provide the sum of $120.00 per annum for secretarial and book-keeping assistance.

Signed

JAMES B. MURPHY, *Chairman*
Executive Committee
W. U. GARDNER, *Secretary*
Executive Committee

Abstracts

Experimental Research, Animal Tumors

Experimental Brain Tumors. II. Tumors Produced with Benzpyrene. ZIMMERMAN, H. M., and ARNOLD, H. [Yale Univ. Sch. of Med., New Haven, Conn.] *Am. J. Path.*, **19**:939-955. 1943.

Twenty-eight tumors developed in the 47 C3H mice implanted with pellets of 3,4-benzpyrene in the right cerebral hemisphere. Fourteen of these were gliomas, 9 were intracranial fibrosarcomas, 2 were extracranial fibrosarcomas, 2 were extracranial rhabdomyosarcomas, 1 was a mixed glioma and sarcoma. The types of glioma were: astrocytoma, ependymoblastoma, glioblastoma multiforme, medulloblastoma, and oligodendroglioma. The gliomas were infiltrative and poorly demarcated from the brain tissue, while the intracranial sarcomas were sharply circumscribed. Subcutaneous transplantation of the intracranial tumors proved valuable as an aid in their study. Three plates of figures illustrate the growths.—J. G. K.

The Spectrographic Analysis of Carcinogenic Hydrocarbons and Metabolites. IV. The Elimination of 1,2,5,6-Dibenzanthracene from the Rat. JONES, R. N., DUNLAP, C. E., and GOGEK, C. J. [Queen's Univ., Kingston, Canada; Harvard Univ., Cambridge, Mass.; and Harvard Med. Sch., Boston, Mass.] *Cancer Research*, **4**:209-217. 1944.

Spectrographic analyses were made of the organs and excreta of 9 rats, each of which received an intraperitoneal injection of 10 mgm. of dibenzanthacene dissolved in tricaprylin. After 14 days the absorption of the hydrocarbon from the peritoneal cavity was almost complete. The feces, collected at 48 hour intervals, contained less than 5.4 mgm. of dibenzanthracene and about 1.5 mgm. of 4′8′-dihydroxydibenzanthracene. Only traces of hydrocarbon or phenolic metabolite were excreted in the urine, and 25% of the hydrocarbon remained in the carcasses in an unchanged condition after 14 days. The phenolic metabolite was detected only in the excreta. The greater part of the hydrocarbon was not accounted for and was presumbly metabolized to some other product that was not detected by the analytical methods employed.—Authors' abstract.

The Spectrographic Analysis of Carcinogenic Hydrocarbons and Metabolites. V. The Elimination of 1,2,5,6-Dibenzanthracene from the Rabbit. JONES, R. N., DUNLAP, C. E., and GOGEK, C. J. [Queen's Univ., Kingston, Canada; Harvard Univ., Cambridge, Mass.; and Harvard Med. Sch., Boston, Mass.] *Cancer Research*, **4**:218-226. 1944.

The elimination of dibenzanthracene from the rabbit was investigated under conditions closely similar to those employed in studies of the elimination from the rat, reported previously.

The observation of other workers that the hydrocarbon is excreted in part as a phenolic metabolite, not identical with 4′8′-dihydroxydibenzanthracene, was confirmed.

The metabolism was studied over periods of 4 and 28 days after intraperitoneal injection, and it was shown that the hydrocarbon passed rapidly into the intestinal tract. About 40% was recovered from the intestinal tract and feces, either as the unchanged hydrocarbon or as the phenolic metabolite. After 16 days the rate of excretion fell to very low levels and only traces of the hydrocarbon could then be detected in the carcass or excreta. The elimination is thus much more rapid in the rabbit than in the rat, and the unchanged hydrocarbon and the phenolic metabolite are probably the main forms in which it is eliminated.

The phenolic metabolite was not detected in the liver or gall bladder, and consideration is given to the hypothesis that the phenolic substances are produced by the action of bacteria in the intestinal tract. However, attempts to obtain such phenolic products by the incubation of dibenzanthracene with cultures from the intestinal tracts of rats or rabbits have been unsuccessful.

The possibility is discussed that some relation may exist between the rapid elimination of dibenzanthracene from the rabbit and the resistance which this animal shows to the carcinogenic activity of the hydrocarbon.—Authors' abstract.

The Ascorbic Acid Content of the Liver in Mice. KENNAWAY, E. L., KENNAWAY, N. M., and WARREN, F. L. [Royal Cancer Hosp. (Free), London, England] *Cancer Research*, **4**:245-250. 1944.

The mean concentration of ascorbic acid in the liver was found to be greater in mice of 3 high cancer pure lines (dba, C3H, RIII), and in the males of the low cancer pure line CBA, than in females of the CBA line and in both sexes of the low cancer pure line C57 and of 2 other low cancer breeds (buff MRC and stock).

Other statistically significant differences were found: (a) between the sexes in C3H and CBA strains (higher in males) and in the dba (higher in females); and (b) between older and younger CBA female mice, the ascorbic acid of the liver decreasing with advancing age.—Authors' summary.

Effect of a Low Lysine Diet on the Growth of Spontaneous Mammary Tumors in Mice and on the

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Microfilm copies of such papers here abstracted as are available may be obtained from Medicofilm Service of the Army Medical Library at 25¢ for each complete article, not exceeding 25 pages in length—and 10¢ for each additional 10 pages or fraction thereof. Prepayment is not requested. Remittance may be made with subsequent orders and in such manner as found most convenient. Address—Medicofilm Service, Army Medical Library, Washington, D. C.

N_2 Balance in Man. Kocher, R. A. [Grace Deere Velie Foundation, Carmel, Calif.] *Cancer Research,* **4**:251-256. 1944.

Diets deficient in lysine but adequate in other respects were fed to 2 normal human subjects. N_2 balance was maintained. A similar low lysine diet was fed to normal young female mice and found to arrest or retard growth. This diet was then given to mice of the Marsh strain with spontaneous mammary carcinomas. It was found that tumor growth was retarded or arrested in its early stage for short periods, but rapid growth was resumed when the lysine deficient diet was continued for periods of over 30 to 40 days or started after the tumors had already attained considerable size. The animals all died prematurely of their tumors when the latter ulcerated or reached large size regardless of the diet. It therefore does not appear promising to use low lysine diets in human cancer patients as a therapeutic measure.—Author's abstract.

Mammary Cancer and Mammary Structure in Inbred Stocks of Mice and Their Hybrids. Bittner, J. J., Huesby, R. A., Visscher, M. B., Ball, Z. B., and Smith, F. [Div. of Cancer Biol., Univ. of Minnesota Med. School, Minneapolis, Minn.] *Science,* **99**:83-85. 1944.

Virgin female A strain mice, 9 to 14 months old, did not develop mammary cancer and displayed no hyperplastic nodules in the mammary glands. C3H (subline Z) virgin females of the same age did have hyperplastic mammary gland nodules and developed mammary cancer in 43% of the cases studied. When the 2 strains were crossed the C3H tendency toward a high mammary cancer incidence among virgin females appeared to be inherited as a dominant character. Virgin females among the F_1 hybrids from both C3H female × A male, and A female × C3H male crosses had mammary cancer incidences comparable with that of C3H virgin females and like them displayed hyperplastic mammary gland nodules.

Both the milk agent and hormonal stimulation appeared to be involved in the production of the hyperplastic nodules. Neither the virgin A females, possessing the milk agent but suboptimal hormonal stimulation, nor fostered breeding A and C3H females, having greater hormonal stimulation but no milk agent, developed the nodules.—R. B.

Characterization of an Influence Affecting Growth of Transplantable Leukemias in Mice. Law, L. W. [Roscoe B. Jackson Memorial Lab., Bar Harbor, Me.] *Cancer Research,* **4**:257-260. 1944.

An influence transmitted in the milk of certain lactating female mice was found to be effective in promoting the growth of 2 transplantable lymphoid leukemias and a transplantable myeloid leukemia in normally refractory mice. It is probable that a similar influence, or influences, affects the growth potentialities in refractory mice of 2 other lymphoid leukemias.

The growth in mice of refractory strains, or sub-strains, of 2 fibrosarcomas, a melanoma, a mammary adenocarcinoma, a monocytic leukemia, and 2 other lines of myeloid leukemia were found not to be affected by an influence, or influences, transmitted in the milk.

Saline extracts prepared from homogenized liver, spleen, or mammary gland tissue were found to contain the susceptibility influence of myeloid leukemia, line C1498.

The following characteristics of the susceptibility influence affecting the growth of myeloid leukemia, line C1498, as determined by using mammary gland tissue extracts were found: (a) It will apparently dialyze through parchment paper. (b) It remains stable in 50% glycerin for 30 days at −4° C. (c) A certain heat stability is indicated. (d) The Seitz filter did not remove all of the influence from extracts. (e) Desiccation in vacuum for 4 hours at room temperature apparently inactivated the influence. Desiccation under similar conditions failed to inactivate the influence present in leukemia cells. (f) Digestion, or partial digestion, apparently failed to inactivate the influence.—Author's abstract.

Phenomenon of Local Skin Reactivity to *Serratia marcescens* (B. Prodigiosus). Immunological Relationships between *Serratia marcescens* Culture Filtrates and Shear Polysaccharide. Shwartzman, G. [Mt. Sinai Hosp., New York, N. Y.] *Cancer Research,* **4**:191-196. 1944.

Filtrates of cultures of *Serratia marcescens* in a simple synthetic medium, and their concentrates prepared by Shear and his co-workers are capable of eliciting the phenomenon of local skin reactivity. The phenomenon-producing principles are closely related to or identical with the factors capable of inducing hemorrhage and regression of mouse tumors.

The chemical treatment, including tryptic digestion, employed by Shear and his co-workers for preparation of the product brings about the concentration and purification of the active principles of the phenomenon without inducing any measurable alteration in the antigenic specificity as shown by the immunizing value, the precipitation and the neutralization reactions of the materials.—Author's summary.

Chromosome Complexity in Regenerating Rat Livers. Biesele, J. J. [Univ. of Pennsylvania, Philadelphia, Pa.] *Cancer Research,* **4**:232-235. 1944.

Liver regenerating after partial hepatectomy in adult white rats exhibits the same chromosomal conditions as does control adult liver, not only with regard to average chromosome volume in metaphase and the frequency distribution of average chromosome volumes, but also with respect to the relative proportions of diploid, tetraploid, and octoploid metaphase figures. Therefore the appearance of polytene chromosomes in cancers cannot be a result solely of rapid growth in adult tissues.

Adult liver, both control and regenerating, has modes of average chromosome volume frequency at 0.8 and 1.2 cubic microns, while liver of the newborn rat shows only the smaller sort of chromosomes. The larger chromosomes of adult liver are held not to be polytene, however, because they are only 50% greater in volume than the smaller chromosomes, and because the enlarged diploid hepatic nucleus in the adult does not have an increased number of plasmosomes.—Author's abstract.

Mitotic Incidence in the First 48 Hours of Methylcholanthrene Epidermal Carcinogenesis. Reller, H. C., and Cooper, Z. K. [Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] *Cancer Research,* **4**:236-240. 1944.

This study was undertaken to determine the immediate effect of methylcholanthrene on cell division in the epi-

dermis of the mouse. The inner surfaces of the ears of 24 Swiss mice were painted once with a 0.6% solution of methylcholanthrene in benzene. Ears were removed from each of 3 mice at 6 hour intervals for 48 hours immediately following the application of the carcinogen. Mitotic counts were made on total mount preparations of the epidermis of these ears. Fifteen thousand nuclei were counted in each ear, and the number of mitoses observed was recorded. At 6 and 12 hours after painting the mitotic count was slightly lower than that for normal epidermis, but at 18 hours the mitotic count rose above the normal average and remained above it for the next 30 hours.

It is concluded that methylcholanthrene applied to the skin of the mouse ear produces a stimulating effect on cell division in the epidermis within the first 48 hours after a single application.—Authors' abstract.

The Multiple Constitution of Abnormal Ciliates Produced by Blastomatogenic Agents. MOTTRAM, J. C. [Mount Vernon Hosp., Northwood, Middlesex, and The Radium Inst., London, England] *Cancer Research*, **4**:241-244. 1944.

(1) Measurements of individuals and of micronuclei of normal *Colpidium sp.* and of abnormals produced by blastomatogenic agents showed that the majority were of varying multiple constitution, and a few were singles.

(2) This confirmed previous findings in which, by selection from abnormal races, clones of apparently normal individuals were produced.

(3) The cells of primary tumors are considered from the point of view that the abnormal races of ciliates are equivalent to tumors in multicellular animals; support for this contention is found.—Author's summary.

The Effects of Roentgen Rays on Cell-Virus Associations. Findings with Virus-Induced Rabbit Papillomas and Fibromas. FRIEDEWALD, W. F., and ANDERSON, R. S. [Rockefeller Inst. for Med. Research, and Memorial Hosp., New York, N. Y.] *J. Exper. Med.*, **78**:285-304. 1943.

Irradiation with 5,000 r of roentgen rays caused the regression of virus-induced papillomas of domestic as well as of cottontail rabbits. The regression of the irradiated papillomas was due to pathological changes in the cells rather than to a destruction of the virus, for the amount of virus recovered from the regressing papillomas was equal to, or greater than, the amount extracted from non-irradiated papillomas. However, the fibroma virus of rabbits was much more sensitive to x-ray irradiation both in crude extracts and *in vivo*. While 100,000 r is required to inactivate 50% of the papilloma virus, 10,000 r destroyed at least 90% of the infectivity of the fibroma virus in extracts containing about the same amount of protein.

Irradiation of the papilloma virus or the fibroma virus did not seem to produce any variant.—D. S.

Late Effect of High Voltage Roentgen Rays on the Heart of Adult Rats. LEACH, J. E., and SUGIURA, K. [Memorial Hosp., New York, N. Y.] *Am. J. Roentgenol.*, **48**: 81-87. 1942.

Single doses of roentgen rays varying from 750 to 7,500 r were given to the heart of 17 adult male and female rats of the Sherman strain. No change in the myocardium was observed in animals surviving 68 to 491 days after treatment. There were no late changes in the heart of control animals that received radiation to the lungs or thigh muscle. Pulmonary infection, when present, did not cause any demonstrable change in the heart of these animals. The fluid flow theory of Failla was applied to explain the relative resistance of the myocardium to roentgen irradiation.—J. F.

Studies in Selective Differentiation of Tissues by Means of Filtered Ultraviolet Light. HERLY, L. [New York, N. Y.] *Cancer Research*, **4**:227-231. 1944.

An attempt was made to determine whether different tissues fluoresce in specific colors and whether such specificity of colors can be employed in differentiating malignant from benign lesions. It appeared that the primary fluorescence excited in these tissues by the filtered ultraviolet light used in this study might be employed by the surgeon in the operating room in an examination of the fresh specimen, for the tissues are seen in a variety of colors, without fixation or staining. The ultraviolet radiations were obtained from a lamp consisting of an 85 watt mercury capillary arc enclosed in a protective glass bulb. A filter holder to accommodate the spectral filter was directly in front of the lamp. The filter used permitted the passage of ultraviolet light, absorbing all significant visible rays. Under these conditions the architecture of the tissues under examination becomes sharply outlined. Small nodules, otherwise hardly visible, become clearly defined. Lymph nodes invaded by malignant cells apparently duplicate the fluorescent features of the primary tumor. All varieties of diseases and neoplasms of the breast were examined for their fluorescent properties. Over 200 specimens of pathological breast tissue were examined immediately after removal in the operating room. One error of diagnosis was made with this method as against one made with the method of frozen sections.—Author's abstract.

Hypertrophic Pulmonary Osteoarthropathy Associated with a Bronchiogenic Giant Cell Tumor in the Left Lung of a Dog. POLEY, P. P., and TAYLOR, J. S. [Kingston, N. Y.] *J. Am. Vet. M. A.*, **100**:346-352. 1942.

Report of a case in a 2 year old Pointer.—E. E. S.

Cancer of the Thyroid in a Dog. KAPLAN, S. L. [U. S. Army] *J. Am. Vet. M. A.*, **101**:202. 1942.

The dog, a 13 year old Pekingese, died during resection of a poorly encapsulated nodular thyroid tumor. There is no mention of examination of the remainder of the body.—E. E. S.

Reticulo-Sarcoma in a Jersey Cow. PIGMAN, E. G. [Houston, Texas] *J. Am. Vet. M. A.*, **101**:203. 1942.

Autopsy disclosed enlarged lymph nodes in a 7 year old cow, and sections were interpreted as indicated. The spleen was not involved.—E. E. S.

A Change in Color Pattern and Neoplasms in a High Producing Hen. GREGORY, D. W. [North Carolina State Coll., Raleigh, N. C.] *J. Am. Vet. M. A.*, **101**:204. 1942.

A Barred Plymouth Rock hen became emaciated and developed scattered white feathers. Autopsy disclosed ascites and tumor throughout the abdominal cavity and in the liver. A diagnosis of lymphosarcoma was made but

not confirmed histologically. The author believes the changed color pattern resulted from endocrine dysfunction, which in turn was caused by the presence of a neoplasm. Although the ovary was tumorous, it was thought unlikely that the tumor, on the assumption that it was lymphosarcoma, was responsible for the color change since avian ovarian lymphocytomas are very common.—E. E. S.

Thoracic Neoplasm Causes Ascites. Viergutz, H. E. [Detroit, Mich.] *J. Am. Vet. M. A.*, **101**:490-492. 1942.

A thoracic tumor encircling the aorta compressed the vena cava and led to ascites. The nature of the growth was not entirely clear; it was thought to be a malignant epithelial tumor, possibly of teratomatous origin. Numerous metastases were present in the lungs.—E. E. S.

Clinical and Pathological Reports

Diagnosis—General

The Treatment of Tumors by Escharotics. Ackerman, L. V., and Eberhard, T. P. [Ellis Fischel State Cancer Hosp., Columbia, Mo.] *J. Missouri M. A.*, **40**:163-166. 1943.

The use of escharotics, in the form of zinc chloride, in the treatment of cancer has been revived recently by Mohs. The present paper is based on a study of 39 cases previously treated by escharotics. It is concluded that the treatment of cancer by these agents, as generally practiced today, is an unsatisfactory, ineffective, and dangerous method. Except in a few instances, it accomplishes nothing that cannot be equally well or better done by radiation or surgery. In certain cases, it offers a means of local attack when cancer must be eradicated with a minimum sacrifice of normal tissue, and when ordinary methods of treatment are not applicable. In any case, zinc chloride should not be used without the safeguards developed by Mohs. The paper includes case histories and photographs illustrating the damage that can be caused by the injudicious use of escharotics.—A. C.

Radiation—Diagnosis and Therapy

Bone Cyst Successfully Treated with X-Rays. Barden, S. P. [Hosp. of the Univ. of Pennsylvania, Philadelphia, Pa.] *Radiology*, **39**:732-733. 1942.

A case of cystic tumor of a bone of the little finger, successfully treated, is discussed. It is of interest because of the location of the lesion, the length of time it had been present (30 years), its response to irradiation, and the control of pain by nerve section.—A. C.

Cancer of the Larynx—Treatment by Irradiation and Report of Cases. Bulson, E. L. [Fort Wayne, Ind.] *Indiana State Med. Ass. J.* **35**:192-196. 1942.

The author reviews some of the previously held opinions on surgical and roentgenologic treatment of laryngeal carcinoma and discusses the validity of the basis for choice between the two procedures. He believes that the decision as to the radiocurability of a particular tumor should not be based on histologic structure but on the degree of invasion that can be established by the mobility of the tumor and the surrounding structures. The majority of early carcinomas are thought to be operable. The author gives details of 3 patients with well advanced laryngeal cancer without recurrence, 2 years, almost 2 years, and 4 months, respectively, after irradiation therapy.—E. E. S.

Treatment of Leukemia by Radioactive Phosphorus. Craver, L. F. [Memorial Hosp., New York, N. Y.] *Bull. New York Acad. Med.*, **18**:254-262. 1942.

The limitations of both local and spray irradiation of patients with leukemia are presented. In chronic leukemia there is a marked elevation of blood phosphorus, which is increased by radiation. Leukemic tissues pick up radioactive phosphorus in greater concentration than do normal tissues. Since 75% of administered P^{32} has decayed by the end of 3 weeks, there is no danger of unduly prolonged effects. P^{32} may be given orally, intravenously, or by other parenteral routes; the oral route is preferred. Dosage is discussed in detail. Eleven patients with chronic myelogenous leukemia were treated by this method, with improvement in 7 of the 9 who had been observed for some time. It is concluded from observation of 11 patients with chronic lymphatic leukemia that this type responds less favorably. There were no real beneficial effects in 2 cases of acute lymphatic leukemia in adults and in 11 in children. The lymph nodes and spleen had diminished in size in a girl with eosinophilic leukemia.—E. E. S.

Irradiation Therapy of Carcinoma of the Uterine Fundus. Fricke, R. E. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **17**:193-197. 1942.

Treatment offers more hope in carcinoma of the body of the uterus, than in any other internal malignant neoplasm. Here, as in any form of cancer, the earliest possible diagnosis is essential. In early localized lesions, with patients in good general health, major surgical operation followed by irradiation is the treatment of choice. In advanced lesions decided value, palliation and occasional cure result from well-planned irradiation therapy.—J. L. M.

Three and Five Year End-Results in the Treatment of Carcinoma of the Cervix at the American Oncologic Hospital. Hahn, G. A. [American Oncologic Hosp., Philadelphia, Pa.] *Am. J. Roentgenol.*, **48**:195-200. 1942.

Results of the treatment of 142 cases of carcinoma of the cervix seen over a 10 year period are reported. Only those treated by combined x-ray and radium and those with a positive biopsy are included in the report. The Schmitz grouping of cases was used in preference to the League of Nations method as being more logical in its definitions.

Intracavitary radium was first administered in doses of from 2,400 to 3,600 mgm.-hr. and followed, after the reaction had subsided, by about the same dose applied directly to the cervix. Following radium application, deep roentgen therapy was given with 200 kv. to a total of 1,800-2,400 r (measured in air) to each of 4 ports. Recurrences were treated with further roentgen therapy except in cases where the initial dose was large.

The results show a 3 year survival rate of 37% and a 5 year survival of 24% when all grades of tumor are classed together. Among patients classified in groups I and IV the 5 year survival rate was 100% and 10% respectively.—J. F.

Ureteral Obstruction Following Irradiation Treatment of Cancer of the Cervix. HOFFMAN, P. E. [Stanford Univ. Sch. of Med., San Francisco, Calif.] *West. J. Surg.*, **50**:69-72. 1942.

Ureteral obstruction is the most frequent cause of death in patients suffering from stage 3 or 4 cervical cancer. The means at present employed for alleviating this condition have been disappointing and other methods must be considered.—M. E. H.

Roentgen Diagnosis and Treatment of Primary Pulmonary Neoplasm. HOLMES, G. W. [Massachusetts General Hosp., Boston, Mass.] *Am. J. Roentgenol.*, **48**:425-432. 1942.

The author reviews the diagnosis and treatment of pulmonary neoplasm and presents the results obtained at the Massachusetts General Hospital for the 10 year period ending April, 1940.

The diagnosis is essentially a radiological problem with fluoroscopy, radiography with routine over-exposed and Bucky studies of the lungs all playing an important part. Signs of partial or complete bronchial obstruction, abnormal movements of the diaphragm and mediastinal shift are noted as important diagnostic findings. Thoracic exploration and bronchoscopy are stressed as important aids in differentiating between a benign and malignant tumor and in grading the latter.

Three hundred and sixty-three tumors were diagnosed, 155 proved microscopically. Fifty-eight per cent were epidermoid carcinoma; 14.5%, adenocarcinoma; 8.4%, undifferentiated; and 18.7% were "oat-cell" carcinoma.

Treatment is based on the findings mentioned above. Benign and localized malignant lesions are operable and should be so treated, while considerable palliation with prolongation of life can be obtained in some cases by the use of deep x-ray therapy. Five of 27 patients subjected to surgery are living and apparently free of disease, while no 5 year cures were obtained by the use of deep x-ray therapy.—J. F.

Contact Roentgen Therapy. Evaluation of Results from a Clinical and Pathological Standpoint. HOWES, W. E., and CAMIEL, M. R. [Brooklyn Cancer Inst., Brooklyn, N. Y.] *Am. J. Roentgenol.*, **48**:360-376. 1942.

The authors report their experience with the Phillips tube and describe the first 100 consecutive cases treated with it. The tube is of value in treating superficial circumscribed lesions on the surface and in the body cavities.

The advantages and limitations of the method are discussed. The authors present depth-dose tables for various distances and filters and the method of treatment of many cases. The results are comparable to those obtained with higher voltages.—J. F.

The Sphere and Clinical Application of Radiation Therapy. KEITH, D. Y. [Louisville, Ky.] *Kentucky M. J.*, **40**:234-238. 1942.

In this review the application of radiation therapy to skin neoplasms and to carcinomas of various organs is discussed.—J. L. M.

Roentgen Therapy of Pituitary Adenomas. KERR, D. H., and COOPER, W. K. [State Univ. of Iowa, Coll. of Med., Iowa City, Iowa] *Am. J. Roentgenol.*, **48**:467-475. 1942.

Following an extensive review of the literature the authors present 25 cases of pituitary tumor that received x-irradiation and were followed for 1 year or more. Nine cases treated but followed for less than 1 year and 6 that were not treated are not included in the report. Two patients were less than 12 years of age. Seventy-six and six tenths per cent of the tumors were of chromophobe type, 21.9% chromophile, and 2.4% basophile. Seven and three tenths per cent were found at operation to be cystic. The patients were given a tumor dose of 2,000-3,200 r with 200 kv. Results showed that in 56% of the patients improvement was maintained, in 20% the disease was arrested, and in 24% it continued to progress.—J. F.

Roentgen Therapy of Primary Cancer of the Nasopharynx. LENZ, M. [Presbyterian and Montefiore Hosps., New York, N. Y.] *Am. J. Roentgenol.*, **48**:816-832. 1942.

The author presents a review of 63 cases of cancer of the nasopharynx treated by external irradiation between 1926 and 1938. Of 44 patients who had been treated more than 5 years previously 13 were free of disease. In this group there were 6 cases of lymphoepithelioma, 5 lymphosarcomas, 1 undifferentiated, and 1 slightly differentiated, epithelioma. Dosage in those patients who were free of disease after 5 years was only slightly more than in those who were not. The author concludes that roentgen therapy is the method of choice in carcinoma of the nasopharynx.—J. F.

The Layer Technique in Radium Needle Therapy. MARTIN, C. L. [Dallas, Tex.] *Am. J. Roentgenol.*, **48**:377-383. 1942.

The author compares the use of radon seeds with radium needles, preferring the latter because they may be more accurately placed. Details of the technic are given.—J. F.

Total Body Irradiation. With Review of Cases. MEDINGER, F. G., and CRAVER, L. F. [Memorial Hosp., New York, N. Y.] *Am. J. Roentgenol.*, **48**:651-671. 1942.

The authors present the results of the treatment of 270 cases of Hodgkin's disease, lymphosarcoma, lymphatic leukemia, myelogenous leukemia, polycythemia vera, mycosis fungoides, multiple myeloma and numerous cases of carcinoma and sarcoma with generalized metastases. Treatment was administered with the Heublein unit. This operates at 185 kv., 1 ma., 5.5 mm. Cu equivalent filter, at a distance of 3 meters. The output varies from 1.5 to 0.37 r per hour. The patients are treated in hospital rooms specially designed for the purpose so that treatment may continue throughout the day with only necessary interruptions.

The results show that total body irradiation produces the greatest palliation in the group of lymphomatoid diseases, and therefore its usefulness is largely restricted to this group of radiosensitive tumors. Polycythemia vera responded well; mycosis fungoides and multiple myeloma showed less striking response; the cases of generalized carcinoma or sarcoma showed no appreciable improvement.

The authors feel that total body irradiation produces the greatest benefit when combined with local high voltage roentgen therapy to enlarged organs (lymph nodes, spleen). It has resulted in longer intervals of freedom from disease in numerous cases and in better survival rates following treatment.—J. F.

The Radiation Treatment of Cerebellar Medulloblastoma. Report of Thirty-One Cases. PENDERGRASS,

E. P., Hodes, P. J., and Godfrey, E. W. [Hosp. of the Univ. of Pennsylvania, Philadelphia, Pa.] *Am. J. Roentgenol.*, **48**: 476-490. 1942.

The pathologic characteristics of the tumor and the clinical manifestations are presented. The spread of the tumor to other parts of the brain and spinal cord is discussed. The method of x-ray treatment following the limited surgical procedure of decompression is described. The histopathological effects of irradiation show a striking change in the tumor cells, which is more pronounced at the periphery of the growth and less so in the stroma. On the basis of 56 patients the authors feel that the method of treatment is unsatisfactory since no complete cures resulted but point out that the survival rate is increased over that of untreated cases. They plan in the future to repeat the cycle of radiation therapy after 4 to 6 months.—J. F.

Pulmonary Metastasis and Pneumonitis Following Radiation Therapy for Cancer of the Breast. Pendergrass, E. P., and White, G. [Hosp. of Univ. of Pennsylvania, Philadelphia, Pa.] *Am. J. Roentgenol.*, **50**:491-498. 1943.

Fifty-four cases of pulmonary metastasis from cancer of the breast are presented, demonstrating 3 essential types of metastases: (a) nodular, (b) infiltrative, and (c) pleural.

The influence of roentgen therapy given prior to the development of metastasis is discussed. Nodular types of metastasis occur in those patients in whom there has been little or no irradiation to the lungs, while the infiltrative forms are found more often after relatively heavier doses of roentgen therapy to the lung fields.

Bleb formation is described as a possible additional diagnostic sign in irradiation pneumonitis. The use of the term pleuropneumonitis is questioned. The similarity of infiltrative metastases and irradiation pneumonitis is briefly discussed.—E. H. Q.

Experiences with Roentgen Irradiation Following Operation on Brain Tumors. Rowe, S. N., and Jacox, H. W. [Western Pennsylvania Hosp., Pittsburgh, Pa.] *Am. J. Roentgenol.*, **49**:480-486. 1943.

On the basis of 33 cases of cerebral glioma, the authors conclude that the best results are obtained when most of the tumor can be removed surgically, and subsequent intensive irradiation administered without increasing the intracranial pressure. When the tumor is inaccessible surgically, decompression and irradiation may be helpful. Approximately half their patients died within 1 year, regardless of treatment. At present it is impossible to tell beforehand which patients will fail to respond and which may be definitely benefited by treatment, hence it is rarely justified to refuse treatment even to the patient with far advanced disease.—E. H. Q.

Isodose Charts for Fields of Special Usefulness in the Treatment of Cancer of the Uterine Cervix. Silverstone, S. M., Braestrup, C. B., and Wolf, B. S. [Mt. Sinai Hosp., New York, N. Y.] *Am. J. Roentgenol.*, **49**:819-821. 1943.

The use of two anterior and posterior, 10×15 cm., pelvic fields is contrasted with that of single 15×15 cm. fields with a 4 cm. central lead strip. Isodose charts for both beams are given.—E. H. Q.

The Roentgen Treatment of Cancer of the Esophagus. Smithers, D. W., Clarkson, J. R., and Strong, J. A. [Royal Cancer Hosp. (Free), London, England] *Am. J. Roentgenol.*, **49**:606-634. 1943.

Of 115 cancers of the pharynx and esophagus seen at the Royal Cancer Hospital from 1936 to 1939, 66 were treated with 400 kv. x-rays. Of this group, 32 received tumor doses of at least 5,000 r. Only 3 patients are still alive. A detailed account is given of the method of calculating the tumor dose.—E. H. Q.

The Roentgenologic Aspects of Ewing's Tumor of Bone Marrow. Swenson, P. C. [Coll. of Physicians and Surgeons, New York, N. Y.] *Am. J. Roentgenol.*, **50**:343-354. 1943.

Ewing's tumor shows great variation in roentgenologic manifestation as well as in histologic picture. For this reason a definite diagnosis can rarely be made from the x-ray examination alone. In planning x-ray therapy it must be remembered that the disease may spread extensively through the soft tissue of the marrow cavity, without producing visible bone destruction. Treatment by a combination of surgery and radiation is recommended.—E. H. Q.

Fractures of the Rib Cage Following Interstitial Radium Therapy for Cancer of the Breast. Wammock, H., and Arbuckle, R. K. [Jeanes Hosp., Philadelphia, Pa.] *Am. J. Roentgenol.*, **50**:609-615. 1943.

Ten cases are reported of rib fracture following treatment with radium needles for cancer of the breast. These patients are all free of clinical evidence of local or metastatic disease. The fractures usually appeared in less than 2 years after treatment, in one case as early as 11 months.

In the differential diagnosis, traumatic fracture and metastasis must be ruled out. The former can be done on the basis of a careful history, the latter on the x-ray appearance of the ends of the fragments.—E. H. Q.

Nervous System

Oligodendrogliomatosis of the Cerebrospinal Pathway. Beck, D. J. K., and Russell, D. S. [Oxford, England] *Brain*, **65**:352-372. 1942.

The authors describe the clinical course and pathological features of 4 cases of oligodendroglioma of the brain in which dissemination of tumor occurred throughout the cerebrospinal fluid pathways. In 3 instances the primary tumor abutted on the ependymal surface of the ventricles and metastases were found in the ventricular walls as well as in the subarachnoid space of the brain and spinal cord. In the 4th case, the site of origin of the tumor could not be determined with certainty and the ependyma was not involved. Histologically, diffuse mucinous degeneration of the stroma is frequently seen in the oligodendroglioma and is of aid in distinguishing this tumor from other metastasizing gliomas such as the medulloblastoma and spongioblastoma. In agreement with the experience of others, the authors found deep x-ray therapy to be of no benefit in their cases of oligodendroglioma.—E. E. S.

Studies on Headache: The Mechanisms and Significance of the Headache Associated with Brain Tumor. Kunkle, E. C., Bronson, S. R., and Wolff, H. G.

[New York Hosp., and Cornell Univ. Med. Coll., New York, N. Y.] *Bull. New York Acad. Med.*, **18**:400-422. 1942.

The quality and intensity of headache caused by brain tumor are described. Data are presented to show that lesions of remotely separated structures within the skull may cause headache in identical areas. It was found that the pain was unrelated to increased intracranial pressure and could often be shown to be due to traction on local pain sensitive structures. When intracranial pressure was increased in addition, there was both local and distant traction through displacement of the brain or by internal hydrocephalus. Usually failure to develop headache was seen only with the more slowly growing tumors. Pain is an asset as a localizing sign when it results from local traction. It sometimes is the initial symptom of a tumor. In the patients described pain in the back of head and neck was never absent in the presence of an infratentorial lesion; when the pain was solely frontal, it was usually due to a supratentorial tumor. Pain is more often on the side of the lesion. A number of generalizations of aid in diagnosis are presented.—E. E. S.

Acoustic Tumors. With Special Reference to End-Results and Sparing of the Facial Nerve. Nielsen, A. [Neurosurgical Clinic, Serafimerlasarettet, Stockholm, Sweden] *Ann. Surg.*, **115**:849-863. 1942.

This is a review of 130 verified acoustic tumors treated from 1930 to 1939, with an analysis of end-results, particularly emphasizing mortality statistics, fate of the facial nerve, and earning capacity of the surviving patients. Complete extirpation of the tumor is favored rather than subtotal extirpation or intracapsular enucleation. In the group treated by the first method during the last 2 years covered by the report, the mortality rate was 11.1%, the facial nerve was preserved in 65% of the patients, and earning power was complete or only slightly diminished in 75.7%.—E. A. L.

Malignant Degeneration of Neurofibromata of Peripheral Nerve Trunks (von Recklinghausen's Disease). Speed, K. [Chicago, Ill.] *Ann. Surg.*, **116**:81-85. 1942.

In 2 patients malignant degeneration of neurofibromas occurred, and its presence was signified by pain. Early amputation is the procedure of choice.—W. A. B.

Breast

Cancer of the Breast. Buchanan, E. P. [Pittsburgh, Pa.] *Pennsylvania M. J.*, **46**:944-948. 1942-43.

A brief discussion of the operative results in 363 cases of carcinoma of the breast.—J. L. M.

Osteochondrofibrosarcoma of the Breast. Carluci, G. A., and Wagner, R. F. [Bellevue Hosp., New York, N. Y.] *Am. J. Surg.*, **61**:271-276. 1943.

Report of a case.—W. A. B.

Disappearance of Breast Cancer with Stilboestrol. Edwards, R. T. Correspondence. *Brit. M. J.*, **2**:659. 1943.

Edwards reports the following in a letter to the Editor. A typical case of scirrhous carcinoma (in a married woman aged 62) was treated by radical mastectomy. The pathological report was: "Carcinoma of breast; growth is a spheroidal-celled carcinoma; there is one large deposit of similar growth in the axilla." Removal of the axillary deposit proved incomplete, and 5 months later there was also extensive recurrence in the skin, scapular region, and posterior triangle. It was decided to administer stilbestrol. Under this treatment, the patient's health gradually improved, and the tumors slowly disappeared, until at the end of a further 3 months regression appeared to be complete. At this time the patient had gained 16 lbs. in weight, and the hemoglobin value was estimated to be 95% (as against 76% initially). The dosage of stilbestrol was 0.5 mgm. daily for 3 periods of 24 days.—A. H.

Frequency, Clinical Course and Treatment of Metastases from Cancer of the Breast. Freid, J. R., and Goldberg, H. [New York, N. Y.] *Am. J. Roentgenol.*, **50**:499-511. 1943.

In 641 patients with cancer of the breast, 160 showed local recurrence after radical mastectomy. These patients had no x-ray therapy to the operative field. These lesions may arise by direct invasion from the primary tumor, by lymphatic permeation, by lymphatic or blood emboli.

Among 168 patients, 81 had skeletal metastases. Of these, 13 showed no other metastases, while the remainder had growths in other regions. Most of the skeletal metastases were multiple.

Of 131 patients coming to autopsy, 117 showed involvement of lungs, pleura, or mediastinal lymph nodes. The majority of these also had involvement in one or more abdominal viscera.

Protocols of 369 patients were reviewed for evidence of metastases to the central nervous system—such evidence was recorded for 89. The metastases were verified for the 40 patients examined post mortem.

Radiation therapy is shown to be useful in controlling skin metastases and in producing palliation of symptoms in skeletal growths and in those of the central nervous system. It is of little value in other metastases. Castration is of value especially in bone lesions, although improvement in other involved regions may also occur.—E. H. Q.

Differential Diagnosis of Breast Tumor. Ryan, J. A. [Covington, Ky.] *Kentucky M. J.*, **39**:32-36. 1941.

Some of the common forms of breast tumors are listed, and brief mention is made of the problem of their differential diagnosis.—J. L. M.

The Anterior Pituitary Gland in Women with Carcinoma of the Mammary Gland, with Report of a Case of Chromophobe Adenoma. Steiner, P. E., and Dunham, L. J. [Univ. of Chicago, Chicago, Ill.] *Am. J. Path.*, **19**:1031-1042. 1943.

Differential cell counts, made on the anterior pituitary lobes of 12 women with mammary carcinoma and of 15 women with tumors of other types, revealed no significant differences in the percentages of chromophobes, acidophils, or basophils in the 2 groups. Although recognizing the possibility that the human pituitary gland may not respond to estrogens in the same way as does that of rodents, the authors consider that there was no evidence of hyperestrogen effects in the 12 breast tumor cases. In a thirteenth case with mammary carcinoma, however, an adenoma of chromophobe cell type was

found, which might possibly be considered as evidence for a hyperestrogen effect.—J. G. K.

Female Genital Tract

Pseudomucinous Cystadenoma with Malignant Changes. Mitchell, R. S. [Wenatchee, Wash.] *Northwest Med.*, **41**:27. 1942.

The tumor occurred in a 12 year old girl. Although it grew slowly over a period of 5 to 6 months, histologic examination after resection revealed the presence of malignant tissue. The child died 2 months later after having developed signs of metastatic growths in the lungs and pleura.—E. E. S.

Cancer from a Gynecologic Viewpoint. Randall, J. H. [State Univ. of Iowa, Coll. of Med., Iowa City, Iowa] *J. Iowa M. Soc.*, **32**:166-169. 1942.

The author reviews 744 cases of cancer of the female genital organs, seen at the University Hospital, Iowa City, from July, 1926, to July, 1936.

Of these 65% were carcinoma of the cervix; 16%, of the body of the uterus; 11%, of the ovary; 3%, of the vulva; 2%, of the vagina; 1%, sarcoma of the uterus; 1%, chorionepithelioma; and 1%, carcinoma of the tube. Each of these types is discussed briefly in regard to symptoms, diagnosis, and treatment.—J. L. M.

Adenocarcinoma of the Cervix. Simpson, B. T., Thibaudeau, A. A., and Burke, E. M. [State Inst. for the Study of Malignant Diseases, Buffalo, N. Y.] *New York State M. J.*, **42**:767-769. 1942.

Adenocarcinoma of the cervix is a relatively infrequent tumor. From 4 to 7% of all malignant growths arising in the cervix are placed in this group by various investigators. A survey of the patients presenting themselves at the State Institute for treatment of cervical cancer shows that the ratio of adenocarcinoma to squamous-cell carcinoma was 1 to 26. Sixty-three patients with cervical adenocarcinoma were selected for study. The duration of the disease is closely allied with the histologic group to which the tumor belongs. While the clinical grouping is the paramount factor in considering the prognosis, the histologic grade had a definite bearing on the end result. In this series women who had not borne children were found to have adenocarcinoma of the cervix in a higher proportion than is usually reported. Thirty-eight per cent survived for 5 years under treatment that consisted mainly of radiation therapy.—J. L. M.

How Shall We Treat Fibroids of the Uterus? Vogt, W. H. [St. Louis, Mo.] *J. Missouri M. A.*, **39**:207-209. 1942.

Modern treatment of this condition is expectant, surgical, or radiologic, and a correct decision as to the type of treatment for the particular case depends on an intelligent appreciation of the pathological condition and the clinical behavior of these tumors. The author discusses the circumstances in which each of these treatments should be used.—J. L. M.

Leiomyosarcoma of the Uterus. Wheelock, M. C., and Warren, S. [New England Deaconess Hosp., Boston, Mass.] *Ann. Surg.*, **116**:882-890. 1942.

A review from the pathological standpoint of 35 cases of leiomyosarcoma.—W. A. B.

Male Genital Tract

Sarcoma of the Prostate. Beatty, R. P. [Uniontown, Pa.] *Pennsylvania M. J.*, **46**:915-918. 1942-43.

Sarcoma of the prostate is briefly reviewed, and 3 cases are reported.—J. L. M.

Carcinoma of the Prostate. Report of an Unusual Case. Buckert, W. I., and Klinger, H. M. [Geisinger Memorial Hosp., Danville, Pa.] *Urol. & Cutan. Rev.*, **47**:207-209. 1943.

An instance of rapidly fatal prostatic carcinoma with metastases in a 29 year old male is reported.—V. F. M.

Clinical Observations on Estrogenic Therapy in Prostatic and Bladder Carcinoma and Benign Prostatism. Haines, W. H., and Miceli, S. [Philadelphia, Pa.] *Pennsylvania M. J.*, **46**:1025-1028. 1942-43.

Four of six patients with prostatic carcinoma were benefited by castration with or without estrogenic therapy. Insufficient criteria have been developed to predict which cases will respond to castration and estrogenic therapy, although the metastatic ones seem to do best.—J. L. M.

Mixed Leiomyoma and Lymphangioma of the Epididymis. Malisoff, S., and Helpern, M. [Beth David Hosp., New York, N. Y.] *J. Urol.*, **50**:104-109. 1943.

A case of a lymphangioma with leiomyomatous features in the epididymis is reported. The authors discount trauma as a causative factor. Both lymphangiomas and leiomyomas have rarely been reported, but one author (Halpert) has previously recorded a mixed leiomyoma and lymphangioma like the one here described.—V. F. M.

Urinary System—Male and Female

Exstrophy of the Bladder Complicated by Adenocarcinoma of the Bladder and Renal Calculi. A Report of a Case and a Review of the Literature. Abeshouse, B. S. [Sinai Hosp., Baltimore, Md.] *J. Urol.*, **49**:259-289. 1943.

Exstrophy of the bladder and vesical adenocarcinoma are discussed at length. Both conditions are rare and concomitant occurrence is extremely so. Twenty-seven instances, including the one here reported, may be found in the literature. Adenocarcinoma of the bladder arises from cystitis cystica and glandularis which in turn have arisen from cell nests of Brunn in association with chronic irritation. Adenocarcinoma of the exstrophic bladder does not arise from misplaced embryological tissue, except in the case of some tumors in the region of the urachus.—V. F. M.

Wilms' Tumor. Daw, W. J. [Wilkes-Barre, Pa.] *Pennsylvania M. J.*, **46**:1293-1295. 1942-43.

Wilms' tumor, a highly malignant renal neoplasm in children, must be diagnosed and treated early if the patient is to have the chance of a cure. Intermediate transperitoneal nephrectomy with postoperative irradiation, or preoperative and postoperative irradiation with nephrectomy, are the methods of procedure.

Four cases of Wilms' tumor are presented with one apparent cure 1½ years after operation.—J. L. M.

Unrecognized Renal Tumors: A Study of 54 Cases in 6,577 Autopsies, and Personal Cases. Hale, N. G., and Burkland, C. E. [Sacramento, Calif.] *J. Urol.*, **49**:426-431. 1943.

This is a report of 54 instances of clinically unrecognized, or "silent," renal tumors taken from the autopsy

files of the Ancker Hospital, St. Paul, Minnesota. The lesions varied from 1 cm. in diameter to large masses. Seventeen had metastasized, but only one tumor that metastasized was less than 8 cm. in diameter. None of the patients had all of the classical symptom triad: hematuria, pain, and mass. Forty-four had not even one of these indications.—V. F. M.

Extratesticular Chorioepithelioma in a Male Probably Primary in the Urinary Bladder. Hyman, A., and Leiter, H. E. [Mt. Sinai Hosp., New York, N. Y.] *J. Mt. Sinai Hosp.*, **10**:212-219. 1943.

Chorionepithelioma rarely occurs in the male, and when it does, with very few exceptions it arises in the testis. It is a highly malignant growth, and the prognosis is very grave, few patients having lived over 2 years. There are only 10 cases of extratesticular chorionepithelioma recorded in the literature. To these the authors add another. This represents the fourth case from this hospital and the third in which the bladder was probably the primary focus. In all 4 instances the biologic tests for gonadotropic hormone were positive. In the case being reported, gynecomastia and pulmonary metastases were present. Bilateral orchidectomy and roentgen-ray therapy had no beneficial effect on the tumor. Serial microscopic sections of both testicles failed to reveal any abnormality, scars, or primary focus in the testicle.—A. Cnl.

Kidney Tumors. Kaufman, I. [Dubuque, Iowa] *J. Iowa M. Soc.*, **33**:114-116. 1943.

A case of malignant nephroma is reported with a brief general discussion of tumors of the kidney.—A. C.

Report of a Case of Wilms' Tumor in an Adult. Loeb, M. J. [Bronx Hosp., New York, N. Y.] *J. Urol.*, **50**:268-273. 1943.

This is a well illustrated report of a single case of Wilms' tumor in a 49 year old female.—V. F. M.

Oral Cavity and Upper Respiratory Tract

Gum Tumours in Pregnancy and Gingivitis Gravidarum. Cross, W. G. [Royal Air Force Volunteer Reserve, Medical Branch] *Brit. Dental J.*, **75**:85-89. 1943.

Gingivitis of pregnancy is regarded as probably due to a combination of factors, the most important of which are vitamin C deficiency, hormonal alterations, and trauma. Of the various forms, the so called "pregnancy gum-tumours" (*epulides gravidarum*), are of special interest; two new cases are described. These tumors are usually single, often pedunculated, grow to a size of 1 cm. or more in diameter, and arise most commonly on the buccal aspect, in the maxilla, and in the lateral incisor-canine region. They appear about the third month of pregnancy, are at their maximum at the seventh or eighth month, and usually disappear rapidly after delivery. There may be an associated gingivitis, but local irritation is often absent.

Treatment suggested is antenatal dental prophylaxis and the administration of large doses (100 to 300 mgm. daily) of ascorbic acid. Removal of gum tumors of pregnancy is not indicated, on account of their prompt regression after parturition.—A. H.

Excision of Amyloid Tumor of the Larynx and Skin Graft: Report of Case. Figi, F. A. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **17**:239-240. 1942.

Heretofore, the treatment of this condition has consisted in destroying the prominent portions of the mass with diathermy and irradiation in an attempt to shrink the tumor sufficiently to restore a free airway. Repeated treatments during the course of months have been required, and results frequently have been indifferent. Surgical removal and skin graft, as carried out in the patient whose case history is reported here, are marked improvements over previous methods of treatment. Since the condition progresses slowly, treatment of this type should offer an excellent chance for permanent relief.—J. L. M.

The Surgical Treatment of Cancer of the Larynx. Jackson, C. L., and Norris, C. M. [Philadelphia, Pa.] *Pennsylvania M. J.*, **46**:822-828. 1942-43.

The surgical indications and the technic of laryngofissure are discussed in this review of the surgical treatment of cancer of the larynx. The authors briefly summarize their experience in a series of 30 consecutive cases in which they had performed laryngectomies by a "narrow-field" technic during a period of about 2 years, without operative mortality or serious complication. Recurrences or metastases had developed in 4 cases and were being treated.—J. L. M.

Extramedullary Plasma Cell Tumor of a Tonsil with Metastasis. McNamara, W. L., and Rogers, R. J. [Veterans Administration Facility, Hines, Ill.] *Arch. Path.*, **36**:89-90. 1943.

Case report.—J. G. K.

Epithelioma of the Lip Metastatic to the Vertebra. Report of Two Cases. Tyler, A. F. [Omaha, Nebr.] *Am. J. Roentgenol.*, **48**:76-77. 1942.

Two cases of epithelioma of the lip with metastases to the vertebra are reported. The author points out that carcinoma of the lip rarely shows bone metastases and states that vertebral metastases, so far as he could ascertain, have never been previously reported. Each case showed compression of 1 vertebra, first lumbar and tenth dorsal respectively, as demonstrated roentgenographically. No autopsy is reported. Biopsy showed squamous cell epithelioma in 1 case only. Both patients had extensive involvement of the cervical nodes.—J. F.

Mixed Tumors of the Lip. With the Report of a Case. Warshaw, D. [New York, N. Y.] *M. Rec.*, **154**:87-90. 1941.

The lip is one of the less common sites of mixed tumor, which usually arises in or near the salivary glands. In a series of 422 neoplasms of this type recorded in the literature, 9 were lip tumors. The mixed tumor reported in the present paper was on the upper lip of a woman of 35 years and was encapsulated. The nodule shelled out readily, local anesthesia being used. Various theories of development are discussed. It is thought most likely that these tumors represent accidental sequestration of embryonal cells. The majority of the tumors are slow growing. There is occasional recurrence. Surgical removal is recommended; roentgen radiation is used when the histologic picture suggests malignancy.—E. E. S.

Salivary Glands

Oncocytoma of the Parotid Gland. Ackerman, L. V. [Ellis Fischel State Cancer Hosp., Columbia, Mo.] *Arch. Path.*, **36**:508-511. 1943.

Report of a characteristic case in a man of 76 years, with 2 figures.—J. G. K.

The Pathogenesis of Mixed Tumors of the Salivary Gland Type. Hemplemann, L. H., Jr., and Womack, N. A. [Washington Univ. Sch. of Med., Barnes Hosp., and Barnard Free Skin and Cancer Hosp., St. Louis, Mo.] *Ann. Surg.*, **116**:34-42. 1942.

Theories concerning the fundamental structure of mixed tumors of the salivary gland type have been based on histologic features. The differentiation between "epithelium" and "stroma" in many areas may be sharp, but in other parts there is gradual, apparent transformation of "epithelium" into "myxomatous" tissue. Histochemical investigation of epithelial and mesodermal mucoids has shown that they can be differentiated by a titration method utilizing the difference in affinity of the protein complexes in the mucoids for dilute aqueous solutions of metachromatic dyes (toluidine blue or polychrome methylene blue). Another method of differentiating the mucoids is based on the greater resistance of the mesenchymal mucoids to hot acids.

The mucoid in the myxomatous and cartilaginous areas in mixed tumors of the salivary glands behaves as does the chondroitin sulphuric acid complex in skeletal cartilage, chondromas etc., while the mucoid in the acini stains exactly as the mucoprotein complex in the mucin of the salivary gland, gastrointestinal and respiratory tracts stains. It is considered that the cartilaginous and myxomatous areas in mixed tumors of salivary glands are truly mesodermal and that the "epithelial" elements are truly epithelial. The pathogenesis of these tumors is presumed to be on the basis of embryonic alteration in tissue relationships in accordance with the "organizer theory" of Speeman.—W. A. B.

Adenolymphoma of the Parotid Gland. Plaut, J. A. [Yale Univ. Sch. of Med. and New Haven Hosp., New Haven, Conn.] *Ann. Surg.*, **116**:43-53. 1942.

All reported cases (48) of this relatively rare tumor are reviewed, and 19 previously unreported cases are presented. These tumors comprise much less than 10% of all parotid tumors and with rare exceptions are benign (96.7%). They occur usually in the fifth, sixth, and seventh decades of life, are 5 times more common in males than in females, and are believed to arise from the growth of parotid tubules and acini that have been found within lymph nodes adjacent to the parotid gland. The treatment of choice is surgical extirpation.—W. A. B.

Intrathoracic Tumors—Lungs—Pleura

Intrathoracic Tumors in Children: Successful Operations in Three Cases. Harrington, S. W., and Logan, G. B. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **17**:363-365. 1942.

Intrathoracic tumors do not occur commonly in childhood. Of those that are seen many are not amenable to surgery. The authors report 3 cases that are of interest for several reasons, not the least of which is that the patients are living and well 6 to 14 months after operation.—J. L. M.

Carcinoma of the Lung. Johnson, H. E., and Daniel, R. A., Jr. [Vanderbilt Univ. Sch. of Med., Nashville, Tenn.] *J. Tennessee M. A.*, **35**:426-432. 1942.

A brief discussion of various aspects of the condition. The high and increasing incidence of this tumor is noted. Emphasis is placed on the mild and insidious nature of the early symptoms leading to erroneous diagnosis until the disease has progressed. Pneumonectomy is regarded as the therapeutic measure of choice.—E. E. S.

Bronchiogenic Carcinoma. Editorial. Major, R. C. [Atlanta, Ga.] *South. Surgeon*, **11**:52-58. 1942.

A general discussion of all aspects of the disease including types, location of the lesion, age of patient, sex incidence, clinical symptoms, physical signs, fluoroscopic and roentgenographic findings, biopsy of primary tumor or cervical node, and treatment. Contra-indications to exploration are listed in detail.—E. E. S.

Bronchiogenic Carcinoma with Metastasis to the Brain. Mattice, E. [Veterans Admin., Hot Springs, S. D.] *M. Bull. Vet. Admin.*, **20**:222-224. 1943.

A case report of interest because the usual signs of cerebral involvement, *e.g.*, choked disc, were absent. Disturbance of olfactory sense, an uncommon symptom, was an early and prominent feature.—M. E. H.

Bronchogenic Carcinoma. McNamara, F. P. [Dubuque, Iowa] *J. Iowa M. Soc.*, **33**:225-227. 1943.

A short general discussion. In the author's series of 178 cases of malignant neoplastic disease 12, or nearly 7%, were proved to be bronchiogenic carcinoma.—A. C.

Some Clinical Aspects of Bronchiogenic Carcinoma. Owen, G. C. [Oshkosh, Wis.] *Wisconsin M. J.*, **42**: 1233-1235. 1943.

Carcinoma of the bronchus is assuming a position of increasing importance in cancer statistics ranking close to carcinoma of the stomach in incidence. Many diseases of the chest produce the same symptoms as those arising from bronchiogenic carcinoma. X-ray and bronchoscopic examination are invaluable aids in establishing the diagnosis.—M. E. H.

Gastrointestinal Tract

Primary Carcinoma of the Duodenum. Berger, L., and Koppelman, H. [Jewish Hosp., Brooklyn, N. Y.] *Ann. Surg.*, **116**:738-750. 1942.

Review of the literature and report of a case.—W. A. B.

Surgical Treatment of Malignant Lesions of the Sigmoid—With Extension. Bowers, R. F. [New York Hosp., and Cornell Univ. Med. Coll., New York, N. Y.] *Ann. Surg.*, **115**:986-995. 1942.

It is suggested that involvement of the bladder by extension of neoplastic lesions of the sigmoid is no reason, in itself, for withholding a radical operation. The author supports this contention by comparing the results in 4 cases in which the diseased portion of the bladder was resected with 2 in which only palliation was attempted. Three patients of the first group were living for periods of from 10 to 46 months postoperatively, and 1 died at 13 months. The outlook in the latter 2 cases was hopeless.—E. A. L.

Hemangioma of the Ileum. CHRISTOPHER, F. [Evanston, Ill.] *Ann. Surg.*, **116**:945-947. 1942.

A case report.—W. A. B.

Transthoracic Resection of Tumors of the Stomach and Esophagus. CHURCHILL, E. D., and SWEET, R. H. [Massachusetts General Hosp., Boston, Mass.] *Ann. Surg.*, **115**: 897-920. 1942.

This paper describes the surgical management of tumors of the lower three-quarters of the esophagus and the adjacent few centimeters of the stomach. Resection of an esophageal carcinoma in the middle half of the organ necessitates complete resection of the thoracic esophagus and a cervical esophagostomy. Of 21 patients reported, 12 had metastases below the diaphragm; esophagectomy was performed on 6 other patients, 3 being alive and well at the time of writing. If the tumor lies in the lower fourth of the thoracic esophagus, in the cardiac orifice of the stomach, or the adjacent few centimeters of the stomach including the fundus, a transthoracic resection is performed with an end-to-side anastomosis between the esophagus and the stomach. Of 21 patients with tumors in this location, resection was done on 13 with carcinoma. Ten patients survived the operation, and 8 are living and well 3 months to 2½ years after operation.—E. A. L.

Transthoracic Resection of Tumors of the Esophagus and Stomach. CHURCHILL, E. D., and SWEET, R. H. [Massachusetts Gen. Hosp., Boston, Mass.] *Ann. Surg.*, **116**: 566-573. 1942.

Brief clinical reports of 24 cases.—W. A. B.

Myo-Epithelial Hamartoma of the Ileum with Intussusception. COX, M. E., and PARKER, E. F. [Med. Coll. of South Carolina, Charleston, S. C.] *Ann. Surg.*, **116**: 355-359. 1942.

A report of a case.—W. A. B.

Extension of the Borderline of Operability in Cancer of the Rectum. DAVID, V. C., and GILCHRIST, R. K. [Presbyterian Hosp., Chicago, Ill.] *Ann. Surg.*, **115**:566-573. 1942.

A group of patients with carcinoma of the rectum, having one or more factors increasing the hazards of the operation, is discussed. It is thought that the indications for the operation should be broadened provided the mortality rate can be kept within reasonable bounds. The mortality was 9.5% in this group of 105 unfavorable cases.—E. A. L.

Surgical Treatment of Carcinoma of the Stomach. EMMETT, J. M. [Clifton Forge, Va.] *South. Surgeon*, **11**:154-163. 1942.

The author believes that too many gastric carcinomas are regarded as hopeless and offers evidence to show that from 18% to 20% of patients could be cured by resection if exploratory operations were promptly performed. Procedures of diagnostic value are discussed.—E. E. S.

Carcinoma of the Stomach with Acute Perforation, Complicated by Bilateral Krukenberg Tumors. Case Report. FRANCIS, J. H. [Memphis, Tenn.] *South. Surgeon*, **11**:498-501. 1942.

The clinical and pathologic features of the case are described.—E. E. S.

The Lymphatic and Venous Spread of Carcinoma of the Rectum. GRINNELL, R. S. [Presbyterian Hosp., and Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] *Ann. Surg.*, **116**:200-216. 1942.

This study is based on the examination of 75 specimens of the rectum and rectosigmoid removed at operation during 1939, 1940, and 1941. Sixty-two were removed by abdominoperineal resection, 10 by perineal excision, and 3 by anterior abdominal resection. Specimens were cleared by the Spalteholtz method slightly modified. The average number of nodes was 52, and metastases were present in 55% of the cases. This is in contrast to 36% found in a similar series of cases from 1916 to 1932 in which the clearing technic was not used. In more than half of the cases with node metastases only 2 nodes or fewer were involved, and these lay within 3 cm. of the tumor. The inadequacy of perineal resection was demonstrated by the presence of involved nodes beyond the limits of possible perineal resection in 13 of 17 cases with metastases in which the tumor lay below the pelvic peritoneum. Lateral spread of carcinoma along lymphatics accompanying the middle hemorrhoidal vessels was found in but 1 instance. Downward lymphatic spread occurs only when extensive metastases have blocked the other routes causing retrograde lymph flow. No relationship was found between the size of the tumor and frequency of metastases, but lymph nodes were involved in 53% of cases when the tumor was not completely annular and in 71% when it was. Invasion of blood vessels was found in 36% of the 75 specimens studied. The frequency of invasion varied directly with the depth of penetration of the bowel wall by the tumor and inversely with the degree of differentiation of the neoplasm. Of the 30 cases with visceral metastases 90% showed blood vessel invasion.—W. A. B.

Granuloma of the Large Intestine Associated with Amebiasis. LIKELY, D. S., and LISA, J. R. [First Medical Division and Pathological Lab., City Hosp., New York, N. Y.] *New York State J. Med.*, **42**:2322-2323. 1942.

A case of granulomatous tumors of the bowel secondary to amebiasis and causing intestinal obstruction is reported. The recent available literature is reviewed. Most of the cases have been diagnosed as carcinoma. Granulomas can develop in treated and untreated cases. Certain outstanding features should indicate the correct diagnosis. The examination of the stool for amebas after the tumor develops is usually negative. Intensive anti-amebic therapy may result in cure. In the majority of cases intensive therapy was not tried. However, it is generally believed that when the granuloma has reached a well-developed stage it will not respond to anti-amebic treatment. Most of the patients operated upon die of peritonitis. Cancer may develop in some instances.—J. L. M.

Surgical Treatment of Carcinoma of the Lower Portion of the Colon. MAYO, C. W. [Mayo Clinic, Rochester, Minn.] *J. Iowa M. Soc.*, **32**:53-56. 1942.

A general discussion. A brief summary is given of the frequency of the several types of operation that were performed in a series of 350 cases.—J. L. M.

Carcinoma of the Rectum, Rectosigmoid and Sigmoid: Selection of Cases for One-Stage Com-

bined Abdominoperineal Resection. Mayo, C. W., and Schlicke, C. P. [Mayo Clinic and Mayo Foundation, Rochester, Minn.] *South. Surgeon,* **11**:14-23. 1942.

The records of 163 consecutive patients upon whom a one-stage combined abdominoperineal resection had been performed are reviewed and the factors important for both clinical and surgical selection are discussed.—E. E. S.

Non-Malignant Lesions of the Large Bowel. Meffert, C. B. [Cedar Rapids, Iowa] *J. Iowa M. Soc.,* **32**: 12-15. 1942.

The author states that the treatment of diverticulitis should be primarily medical except for the complications, but the treatment of polyps should always be surgical. Once the diagnosis of polyps of the colon is made, surgery is advised to protect patients from the possible development of a malignant growth—J. L. M.

Urologic Complications of Cancer of the Rectum. Mullen, T. F., and Lestrohan, P. [Univ. of California Med. Sch., San Francisco, Cal.] *Ann. Surg.,* **116**:6-18. 1942.

Among 93 patients (60 males and 33 females) subjected to various radical procedures for carcinoma of the rectum in 4 different hospitals, urologic complications occurred in 64% (38 males and 18 females). The anesthesia was spinal in 59, general in 27, and in 1, local. The commonest complications were: retention in 56 patients (64%), cystitis in 28 (33%), pyuria in 30 (35%), and pain or burning in 17 (19%). The complications were due to one or more of four factors: (1) direct injury to the urinary tract; (2) loss of supports of the bladder with postoperative sagging and pooling of urine; (3) the necessity of postoperative catheterization [24 patients (27%) required intermittent catheterization, 36 (41%) had retention catheters, and 27 (32%) voided normally in the first 24 hours]; (4) injury to the nerve supply to the bladder. The parasympathetic nerves are most frequently injured, and this occurs during the perineal stage of the operation. Mixed types of dysfunction occur and are due to various degrees of injury to several of the nerve pathways. Injury or division of the pudendal nerve leads to incontinence.—W. A. B.

A Single Carcinomatous Polyp of the Sigmoid Colon. Nesler, A. B., and Faber, L. [Finley Hosp., Dubuque, Iowa] *J. Iowa M. Soc.,* **32**:72-74. 1942.

While both benign and malignant polyps may arise in any portion of the intestine, the sigmoid colon is most commonly involved. The symptomatology is not distinctive, and because some of the growths, though at first benign, ultimately may undergo malignant change, it is important to keep them in mind as possibilities in patients with vague abdominal symptoms. The case presented is one of a single malignant polyp of the sigmoid that probably had its origin in a benign adenomatous polyp. The case is of interest because it is an example of the well-known fact that not infrequently the symptoms due to metastases rather than to the primary malignant growth cause the patient to consult a physician. However, it is rare that the metastases almost completely replace the liver before medical advice is sought. Probably the patient did have symptoms due to the original polyp, but they were so slight that they were neglected. From the appearance of the polyp it may be assumed that it was present a long time if not actually congenital. Therefore, it can be surmised that for a considerable period the lesion was benign. The hope of cure in this case rested upon its detection and removal during this period before the lesion underwent malignant change and formed metastases.—J. L. M.

Carcinoma of the Stomach with Krukenberg-Type of Ovarian Metastases. Nesler, A. B., Lawrence, J. W., and Faber, L. [Finley Hosp., Dubuque, Iowa] *J. Iowa M. Soc.,* **32**:219-221. 1942.

In addition to being an example of an interesting tumor, this case is noteworthy because the father of the patient died of carcinoma of the stomach, which suggests perhaps a genetic predisposition. The prolonged history indicates a gastric ulcer that apparently underwent malignant change. Although the gastric resection apparently resulted in a cure locally, the ovarian metastases which were not evident at the time of the first operation, precluded a successful final outcome.—J. L. M.

Cancer of the Colon—A Diagnostic Problem. Schroeder, M. J. [New York, N. Y.] *M. Rec.,* **155**:54-55. 1942.

Six patients are briefly described to illustrate the well known observation that malignant tumors may produce few or no symptoms in the early stages of their growth.—E. E. S.

Bone and Bone Marrow

Multiple Primary Hemangioma of the Bones of the Extremity. Ackermann, A. J., and Maynard, S. H. [State Univ. and Crippled Childrens Hosps., Oklahoma City, Okla.] *Am. J. Roentgenol.,* **48**:47-52. 1942.

A case is reported of multiple hemangioma involving large areas in the upper end of the left fibula and both ends of the left tibia of a 15 year old boy. The patient was followed for 10 years. For 6 years no progress of the lesions was apparent. Nine years after the first examination extensive involvement of the tarsal bones and the fourth metatarsal was demonstrated roentgenologically. The extensive bone lesions were accompanied by only moderate disability. A biopsy was made and the case submitted to the bone tumor registry (Reg. No. 1361) where an original diagnosis of osteitis fibrosa was later changed to primary hemangioma.—C. E. D.

Multiple Myeloma. (A Review of the Literature and Report of Five Cases.) Greenberg, R. C., and Frosch, H. L. [Masontown, W. Va., and New York, N. Y.] *West Virginia M. J.,* **38**:340-347. 1942.

There is a brief summary of the clinical history, physical findings, and laboratory data in the 5 new cases.—E. E. S.

Bone Sarcoma. Heles, J. B. [Finley Hosp., Dubuque, Iowa] *J. Iowa M. Soc.,* **32**:549-551. 1942.

Bone sarcoma is generally recognized as a disease of younger persons, but this case in a patient 55 years of age serves to emphasize that it is also encountered in elderly people.—J. L. M.

Hemisection of the Mandible for Recurrent Adamantinoma. Holloway, J. W. [University Hosps. and Western Reserve Univ. Sch. of Med., Cleveland, Ohio] *Ann. Surg.,* **116**:277-281. 1942.

A report of 2 cases.—W. A. B.

Epidermoid Carcinoma of the Tibia. Report of Two Cases. KRAFT, E. [City Hosp., Welfare Island, New York, N. Y.] *Am. J. Roentgenol.*, **50**:602-608. 1943.

Direct invasion of bone by epidermoid carcinoma is rare and occurs mostly in the tibia due to its proximity to the skin. The predisposing ulcers of the leg and draining osseous sinuses may exist for many years and even decades, and a malignant degeneration may therefore be overlooked. Cases of an ulcer carcinoma and a fistula carcinoma are reported. The characteristic clinical and roentgenologic features and the differential diagnosis are discussed.

The prognosis is favorable, even in advanced cases, since distant metastatic lesions have been observed on only 6 occasions. Amputation of the diseased leg is usually indicated unless an early diagnosis allows more conservative procedures. In chronic osteomyelitis, efforts should be made to cure residual active foci. With an increase in secretions and development of pain and hemorrhage, periodic roentgenologic and biopsy studies are advisable to determine the presence of an epidermoid carcinoma.—E. H. Q.

Interpelviabdominal Amputation. Report of Three Cases. LEIGHTON, W. E. [Barnard Free Skin and Cancer Hosp., and St. Louis Univ. Sch. of Med., St. Louis, Mo.] *Arch. Surg.*, **45**:913-925. 1942.

A report of 3 cases. The lesions necessitating operation were: (1) osteochondroma of the ilium, (2) chondrosarcoma of the brim of the pelvis, (3) osteosarcoma of the ischium. A review of the literature and description of the operation are included.—G. H. H.

SPLEEN

Hemorrhagic Cyst of the Spleen. DENNEEN, E. V. [New York, N. Y.] *Ann. Surg.*, **116**:103-108. 1942.

A report of 5 cases.—W. A. B.

Calcified Unilocular Cyst of the Spleen. GALLAGHER, P., and MOSSBERGER, J. I. [Station Hosp., Fort Bliss, Tex.] *Ann. Surg.*, **116**:933-937. 1942.

A case report.—W. A. B.

Cysts of the Spleen. MCCLURE, R. D., and ALTEMEIER, W. A. [Detroit, Mich., and Cincinnati, Ohio] *Ann. Surg.*, **116**: 98-102. 1942.

A case report.—W. A. B.

ADRENAL

Adrenalin-Producing Pheochromocytoma of the Adrenal Associated with Hypertension. Report of Three Cases. KIRSCHBAUM, J. D., and BALKIN, R. B. [Woodlawn Hosp., Cook County Hosp., and Northwestern Univ. Med. Sch., Chicago, Ill.] *Ann. Surg.*, **116**:54-60. 1942.

The 3 tumors were benign and were incidental findings at autopsy.—W. A. B.

Pheochromocytoma with Hypermetabolism. Report of Two Cases. MCCULLAGH, E. P., and ENGEL, W. J. [Cleveland Clinic, Cleveland, Ohio] *Ann. Surg.*, **116**:61-75. 1942.

One of the patients reported on was cured surgically. In the other the tumor was found at autopsy. Hypermetabolism, an outstanding feature in both cases, disappeared in the first after surgical removal of the tumor.—W. A. B.

Non-Hormonal Adrenal Adenoma; Case Report. TIMONEY, F. X. [St. Vincent's Hosp., New York, N. Y.] *J. Urol.*, **49**:654-657. 1943.

The successful removal of a 4,200 gm. adenoma of the adrenal is reported. No hormonal disturbances were present.—V. F. M.

PITUITARY

Acromegaly, Partial Removal of Acidophil Adenoma of Pituitary Followed by Two Pregnancies. JACKSON, I. *Proc. Roy. Soc. Med.*, **36**:356. 1943.

Description of a case.—E. L. K.

The Replacement Method in Surgery of Pituitary Tumors. A New Technic. SELETZ, E. [Cedars of Lebanon Hosp., Los Angeles, Calif.] *West. J. Surg.*, **49**:660-661. 1941.

The new technic is useful in those cases in which edema or increased intracranial tension makes the usual approach impossible unless lobectomy is resorted to.—M. E. H.

Abscess Within the Sella Turcica Simulating Pituitary Tumor: Surgical Cure. SVIEN, H. J., and LOVE, J. G. [Mayo Clinic, Rochester, Minn.] *Proc. Staff Meet., Mayo Clin.*, **17**:497-501. 1942.

Isolated reports of cases of metastatic lesions of the pituitary gland in association with abscesses situated elsewhere in the body have been reported in the literature, but the present case is the first seen at the Mayo Clinic and is reported for that reason.—J. L. M.

PANCREAS

Carcinoma of the Islands of Langerhans with Liver Metastasis Producing Hyperinsulinism. BROWNING, J. S. [Indianapolis, Ind.] *Ann. Int. Med.*, **19**:669-673. 1943.

Case report.—J. G. K.

Acinar Cell Carcinoma of Pancreas: Report of Case in Which Function of Carcinomatous Cells Was Suspected. COMFORT, M. W., BUTT, H. R., BAGGENSTOSS, A. H., OSTERBERG, A. E., and PRIESTLEY, J. T. [Rochester, Minn.] *Ann. Int. Med.*, **19**:808-816. 1943.

The values for activity of lipase and of amylase in the serum were exceedingly high, but the data did not show conclusively whether these phenomena resulted from obstruction of the pancreatic ducts and absorption of the enzymes into the blood stream or from functioning of the acinar cell carcinoma.—J. G. K.

The Association of Carcinoma in the Body and Tail of the Pancreas with Multiple Venous Thrombi. KENNEY, W. E. [Washington Univ. Sch. of Med., and Barnes Hosp., St. Louis, Mo.] *Surgery*, **14**:600-609. 1943.

Carcinoma of the body and tail of the pancreas is frequently associated with multiple venous thrombi (7 of 21 cases). This is not true of carcinoma of the head of the pancreas (no instance among 30 cases, but in 5 cases there was a single thrombosed vein). It is suggested that the tumors, which in every case showing multiple thrombi were of the mucinous type, may secrete an abnormal substance or an undue amount of a normal substance concerned in blood clotting.—W. A. B.

Book Review

THE GENETICS OF THE MOUSE. Hans Grüneberg, with an appendix on the Genetics of Cancer in Mice by Drs. C. C. Little and P. A. Gorer. Cambridge University Press, London. 1943. XII 412 pages, 14 plates, 43 illustrations. Price 30s.

Cancer research has been in need of a comprehensive and authoritative account of the genetics of the house mouse because of the unique position the species has attained as an experimental animal and because of the increasing realization of the importance of genes. Dr. Hans Grüneberg satisfies this need with his book on the genetics of the mouse.

The volume stands as a victory over the difficulties of compiling and evaluating a vast amount of data amid the adverse working conditions of a country at war. The book gives a detailed account of the various mouse traits, the inheritance of which has been analyzed from the turn of the century through 1941. The author's evaluation of the available data reflects his thorough understanding of the field, attained through intimate experience, and his broad outlook is indicated by the suggestions offered for future experimentation. Characteristic of his sound judgment is his recognition of the artificiality of the distinction between "qualitative" and "quantitative" characters, "morphological" and "physiological" characters, and in certain instances between "main gene" and "modifiers."

The discussion of genetic analyses of qualitative differences, comprising the greater portion of the book, is outstanding. The amount of basic information that the study of these traits has yielded is impressive, as is also the portion of this information contributed by the author himself. One cannot read this section without realizing that it is genes that are inherited and not characters. The reader is made particularly conscious of the physiological processes that must link the gene to the resultant character, and of the advantages that this mammalian species affords for the study of such processes. Emphasis is placed also on the relationship of the gene in question to the genetic background.

In comparison with the extensive discussion of qualitative characters, the subject of quantitative differences receives rather meager consideration, possibly less than is justified when the importance of the two types of characters is considered. The brevity of this section, however, is an indication of the difficulties encountered in the analysis of such multiple factor traits. The studies of the few characters that have been analyzed (litter size, body size, and tail rings) are well reviewed.

To medical men the section on the inheritance of resistance to infectious diseases will be of particular interest and of great value, although not more worthy of recommendation than other sections dealing with more fundamental aspects of genetics. The discussion of the induction of genetic changes by means of x-rays is particularly apropos at this time when the question of protective measures against irradiation damage is being so widely considered.

The bibliography is extensive and undoubtedly will prove to be extremely valuable.

Readers of *Cancer Research* will consider the section on the genetics of cancer in mice to be of primary interest. Dr. Little and Dr. Gorer, although separated by the Atlantic and with communication greatly curtailed, have nevertheless succeeded in compiling this information without deviating from their usual high standard of presentation This section is rather brief, with comparatively few source data given, but the salient facts are presented in such manner as to give a complete, coherent picture of the development of our knowledge of the subject through 1941, and those desiring more detailed information will find the bibliography satisfying. Tumor transplantation, spontaneous tumors, and induced tumors receive well-balanced consideration.

The genetics of tumor transplantation is not stressed as much today as it was a decade ago, nevertheless this section will be of value to those who are transplanting tumors for studies other than genetic and are interested in reviewing the genetic background of this subject. Mention is made, however, of some of the more recent investigations by Dr. Gorer, who has approached the problem from an immunological point of view; this work shows promise of contributing further interesting information.

Primary attention is given to the genetics of spontaneous tumors. Mammary cancer, lung tumors, liver tumors, adenoma of the stomach, and nonepithelial growths are discussed separately. Although the authors are firmly convinced that genes are concerned in the development of many of these types of tumors, one senses their general caution against any conclusion of simple single factor inheritance. Another indication of a trend toward a more conservative view than that prevalent in the past is their attitude concerning the question of dominance, which has unfortunately occupied too much attention and at present must be regarded as a minor issue.

Salient features of the extrachromosomal influence, especially on mammary tumors, are well presented. The problem of the milk-borne agent is viewed as one in which not only the variation of the experimental animal in susceptibility to the action of the agent must be considered, but also the possibility of variation in the milk-borne agent, if the agent is a virus.

Induced tumors receive due consideration. There is, of course, no discussion of carcinogenesis by the various compounds further than the mention of the more common ones used in each case. However, the discussion gives a good impression of the contributions made to the cancer problem through the study of induced tumors. The susceptibility to each type of induced tumor is compared with that of the corresponding type of spontaneous tumor. The highest correlation occurs in the case of lung tumors, but a correlation is also displayed by certain leukoses. In the case of other types of tumors, however, there appears to be none.

In spite of the enormous amount of information that the geneticists have contributed to the cancer field, their efforts still have yielded comparatively little definite information on the particular genes involved. This is especially apparent to one who has just finished reading the main portion of the book, which discusses the many characters of the mouse for which specific genes are known. Are the genes that are related to each type of neoplasm single or multiple in number, and if multiple is their action cumulative? Furthermore, in what manner do their effects cumulate? Are the genes that increase susceptibility dominant or recessive to their respective alleles? Answers to such questions are conspicuously absent in this rather complete discussion of tumor inheritance. Explanation for this situation is to be found in the complexity of the genetic action and also of the extrachromosomal factors involved.

Experimental oncologists will find the contribution of Dr. Little and Dr. Gorer of undisputed value as a reference and as a guide to future experimentation. Of even more basic value is the main portion of the book, in which Dr. Grüneberg presents so well the more fundamental information on inheritance that must be comprehended by all who would attain a thorough understanding of tumor development.

W. E. Heston.